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EDITOR'S VIEWPOINT

Harris and Hayes (p. 470) used radioactive iodine uptake and metabolism as the indicator of fluoride effects in rats. Neither acute nor chronic exposure to sodium fluoride affected the uptake of the iodine, its transport through the thyroid acinar cell stage, or its distribution to the blood plasma. If fluorides do affect iodine metabolism, as indicated by other investigators, Harris and Hayes suggest the possibility of chemical alteration of the iodothyroglobulin at points other than the iodine bond. A few opponents of fluoridation have hinted that "governmental influences" would deter investigations of an adverse nature. This study, under U. S. Atomic Energy Commission auspices, by an AEC employee and an Air Force officer had the potentiality of being adverse, although it proved otherwise, but it was neither suppressed nor discouraged.

Bishop, Richardson, and Muhler (p. 478), injecting fluoride compounds intravenously, demonstrated that fluorine is a vasodilator with much longer action than most vasodilators. The action influenced the activity of the fluorine. The authors suggest that these findings present a possible mechanism by which fluorine may stimulate salivary flow. Since increased salivary flow has been suggested as a means by which fluorides may reduce dental caries, and a mechanism for such salivary flow effects now has been proposed, studies of salivary flow in high- and low-fluoride water regions are indicated.

Burnett (p. 489) exposed slabs of dentin to various fluoride compounds and various acids in buffered solutions containing protein. The fluorides affected the solubility and accessibility of the protein portion of the dentin differently, but the reactions were complex. Sodium fluoride was more effective than lead fluoride, calcium and potassium monofluorophosphate, and calcium fluoride, but it should be pointed out that this work was a study of the action of fluorides under extremely artificial conditions and not a test of the caries-inhibiting effects. Moreover, the tests were made only on dentin, not on enamel. Burnett has shown the complexity of the problem.

Burnett (p. 503), in a laboratory study, found that silver nitrate, zinc chloride, and potassium ferrocyanide have no marked effect upon the acid solubility, or upon the accessibility of the organic matrix of dentin after its decalcification. This confirms results obtained, clinically, indicating that these compounds do not inhibit dental caries. Many individuals may use new agents which are claimed to reduce dental caries (or other diseases) before their effectiveness has been established, on the theory that "they may do some good but won't do any harm." Burnett's study indicates that protein coagulants may do harm by liberating more accessible organic residues.

Nizel and Harris (p. 513) ashed a natural diet of corn, milk powder, alfalfa meal, and salt and added 3 parts of this ash to 97 parts of the same natural diet. When 17 hamsters were fed the ash-supplemented diet, they averaged much less caries than 24 hamsters receiving the original diet. How the diet affects caries and what the nature of the protective elements in the ashed diet might be are not explained. The observation is interesting but represents only a preliminary survey.

Lilienthal (p. 516) credits the bicarbonate system of saliva with the major role in buffering by saliva. Phosphates appear to contribute somewhat but mucoids are assigned no importance in this process. In the dentobacterial plaque, the microorganisms appear to make the major contribution. Since the pH of the region is accepted as having a role in dental caries and in calculus formation, and, since the plaque and the saliva have different buffering systems, further investigation of the relationships of these two systems may prove fruitful. Perhaps, dominance of one system over the other may, in part, determine whether an area will be covered by calculus or decalcified by acid.

Shafer and Muhler (p. 531) observed reduction in activity (proteolytic and amylolytic) of the salivary glands after hypophysectomy in the rat. There was also an increase in the number of carious lesions

and in the severity of the cavitation in the operated animals. Whether these phenomenon are related or coincidental remains to be demonstrated, but the implication is strong since histologic and functional changes in the salivary glands are demonstrated by Shafer and Muhler and the relation of reduced salivary activity to caries has been well documented.

Using the in vitro conditions of the "artificial mouth," Pigman, Hawkins, Watson, Powell, and Gaston (p. 537) found that the concentration of D-glucose in a bacteriologic medium mixed with saliva and flowed over teeth influenced the effect on the enamel and dentin. At higher glucose concentrations decalcification occurs, while at lower glucose concentrations previously decalcified dentin is softened, pigmented, and destroyed. The bacterial flora of the teeth differed at the different glucose concentrations, streptococci having the maximum numbers at a glucose concentration which was minimal for survival of lactobacilli. The study of the changing flora under these artificial conditions offers an opportunity for learning more of the life cycle of the salivary flora.

Savara (p. 546) reports the incidence of dental diseases in 2,774 children of 14 to 17 years of age. The average number of surfaces affected by caries (DMF) was 19. While one can visualize this, it is more difficult to grasp the significance of 4 PMA units (papillae, margins, attached gingiva) affected per child. These could be traumatized, infected, necrotic, hyperplastic, or otherwise involved. Moreover, one affected papilla is used as evidence of gingivitis, 39 per cent of the children having one or more papillae involved. Malocclusion was found in 77.2 per cent of the children. It is interesting that this compares favorably with 74 per cent reported for freshmen college students (A. Resnick, Thesis, Ohio State University, 1955), and the data for high school students in St. Louis (St. Louis Dental Society, 1933). Where the gingivitis index was high, the caries and malocclusion rates were higher. Which might be cause and which might be effect are not discussed.

Bennett and Miles (p. 553) used bacteriologic methods to study the penetration of dentin, cementum, and enamel by penicillin. Under the in vitro conditions of their experiments cementum and enamel were impermeable and dentin permeable to penicillin in solution. The impermeability of cementum suggests that penicillin placed in pulp chambers can reach the periodontal membrane only through the root canal openings. This is also the way in which pulpal toxins and bacteria reach the periodontal membrane.

Waerhaug (p. 563), on the basis of in vitro studies of the penetration of the gingival sulcus and gingival pocket by dyes, concludes that the mineral salts of subgingival calculus come from the blood. While this is an interesting study, the process of calculus formation may be much more complex than that indicated by simple diffusion of dyes from the mouth. A reawakening of interest in calculus formation is needed and the entire process should be established. This small segment in the study of what may be a complex system has a place, but final conclusions should await more precise knowledge.

Butcher and Vidair (p. 569) subjected extracted teeth of monkeys to such insults as pulp removal, burring, and immersion in formalin or hot water. The teeth were then replanted and show reattachment of the periodontal membrane fibers to new formed cementum. In some regions reattachment and ankylosis alternate. Epithelial proliferation is seen in regions of inflammation, but the authors could not decide whether epithelial downgrowths can cause loss of fiber attachment or whether the fibers must be lost before the epithelium proliferates. This is probably the most severe treatment reported to date with microscopic evidence of reattachment of connective tissue fibers.

Rose, Lal, Williams, and Falcetti (p. 577) tested various materials in an effort to find a suitable substance to bond to tooth structure. Adhesion to dentin varied from that to enamel. Unfortunately, no material available for testing maintained adhesion after prolonged exposure to moisture. One phase of the study indicated that bacteria can lodge in the space between the filling material and the tooth substance.

Thermal expansion and water sorption of resins have been among the chief objections to direct resin restorations. Rose, Lal, Green, and Cornell (p. 589) have shown that various inorganic fillers can reduce both the thermal expansion and water sorption of these materials. Continued search for better filling materials is important, for one of the chief methods for controlling caries will continue to be restoration of carious teeth.

Phillips, Schnell, and Phillips (p. 597), continuing their work on electroconductivity of dental cement, studied the effect of increased contact area and thickness for resins, calcium hydroxide, zinc oxide, eugenol, and zinc phosphate cement. As they point out, their data are based upon observations outside the mouth and it is not yet certain whether these can be duplicated in the mouth. With the exception of calcium hydroxide paste, the substances they studied were excellent insulators when maintained in the dry condition, but exposure to moisture entirely changed the situation. The studies which are made in the mouth certainly will give us further information on the so-called insulating properties of cement and cementlike materials.

Mohammed and Schour (p. 608) have utilized the growing incisor of the rat to study the effects of filling materials. Zinc-oxide–zinc-sulfate cornstarch bases were compared with paraformaldehyde. As observed earlier in other animals, cavity depth is a factor in pulp irritation. The response was minimal in zinc oxide–filled cavities, while severe degenerative changes and necrosis occurred under 1 per cent paraformaldehyde. The rat incisor is recommended as a tool for evaluation of filling materials.

Bass (p. 621) found *Leptothrix racemosa* in 76 of 100 open cavities in permanent teeth. This organism presents somewhat of a problem in classification because it has not been cultured. The entire problem of the filamentous organisms, including leptothrix, actinomycetes, and the "brush" organisms *(L. falciformis)*, are an interesting group and an area requiring more fundamental study. Bass, wisely, avoids attaching any significance, pro or con, to *L. racemosa* in relation to dental caries.

H. B. G. R.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH
1956 MEETING

The next general meeting will convene at the Jefferson Hotel, St. Louis, Missouri, on Thursday, Friday, and Saturday, March 22, 23, and 24, 1956. Please note the *change* in meeting days. The Dental Materials Group of the Association will meet on Friday, Saturday, and Sunday, March 23 to 25. This is the same plan used in preceding years. The American Association of Dental Schools will meet on Monday, Tuesday, and Wednesday, March 26 to 28, also at the Jefferson Hotel in St. Louis.

The Novice Award will be offered again under approximately the same conditions as last year.

Further details will be published later, but prospective participants in the oral presentation program should be prepared for a December 1, 1955, closing date.

Address all communications with reference to this meeting to:

Dr. Dan Y. Burrill, Assistant Secretary-Treasurer
129 East Broadway
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A TRACER STUDY OF THE EFFECT OF ACUTE AND CHRONIC EXPOSURE TO SODIUM FLUORIDE ON THE THYROID IODINE METABOLISM OF RATS

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INTRODUCTION

THE addition of small amounts of fluorine to municipal water supplies as a possible means of mass dental caries control, although favorable from a strictly dental viewpoint,[1, 8, 9, 12] has precipitated considerable controversy because of the possibility of adverse systemic reactions.[5, 10, 16, 22] In the latter category, the possible correlation of the intake of this trace element to thyroid pathology has elicited considerable interest. Following experimental dosage with sodium fluoride, synergistic,[23, 24, 25, 33] inhibitory,[13, 18, 27, 32] and neutral effects [6, 11, 31] upon thyroid physiology have been reported.

Several investigators have found histopathologic changes of the thyroid in fluorine studies, suggestive of an inhibition of thyroid function.[18, 27] Such findings led Goldemberg[13] to postulate that goitrous conditions might be traced to sodium fluoride intake, and conversely, that the element might be utilized in the treatment of hyperthyroidism.[14, 17, 19] Other investigators, in attempting to duplicate these results, could not secure the same definite thyroid changes.[6, 26]

From a biochemical approach, Baumann and Metzger[2] have suggested the interesting possibility that the thyroid gland has an affinity not only for iodine, but also for other members of the Seventh Periodic Group. To substantiate this, they pointed to several studies utilizing bromine,[20, 21] chlorine,[30] manganese,[28] fluorine,[7] rhenium,[4] astatine,[15] and technetium,[3] in which the thyroid consistently demonstrated the ability to concentrate these elements in a ratio of greater magnitude than seen in other tissues of the body. Of greater importance, possibly, is the suggestion that these elements have a mass action effect on the thyroid.

Additional clarification of the role of sodium fluoride in thyroid metabolism is indicated in view of these antithetic reports, and of the theoretical potentialities of inhibiting iodine metabolism. Therefore, the following experiment was designed to elucidate certain aspects of iodine metabolism of the thyroid gland.

The use of the nuclide, iodine-131, was indicated by the fact that any alteration of uptake gradient of the thyroid gland would be readily detected by large variations in the registered disintegration count of the administered radioactive iodine, and by differences discerned in an accompanying autoradiographic study.

This investigation was completed as a portion of the residency program at the Medical Division, Oak Ridge Institute of Nuclear Studies, under contract with the U. S. Atomic Energy Commission.

Received for publication April 14, 1954.

EXPERIMENTAL

Utilizing CF Wistar strain rats, the procedure was divided into 2 experiments.

Experiment I.—This constituted a study to determine the effect that an acute exposure to sodium fluoride would have on the iodine uptake gradient of the thyroid gland.

Ten adult female rats were divided into 2 groups of 5 each. Under ether anesthesia, each rodent of the control group was successively given an intraperitoneal injection of 1 ml. of saline solution containing 250 μc of activity as carrier-free iodine-131. Following this procedure, each was in turn placed in a tunnel of lead bricks, so that only the head and neck protruded. A collimated GM tube was placed directly over the thyroid area at about 1-inch geometry. Gamma radiation counts were recorded at 1-minute intervals for a period of 10 minutes.

The second section of 5 rodents was similarly treated, with the exception that 0.33 ml. of 1.25 per cent sodium fluoride in saline solution was injected intraperitoneally simultaneously with the iodine-131, although on the opposite side of the abdomen.

This simultaneous injection of fluorine along with the iodine-131 produced no significant differences in uptake gradient between the 2 groups, as reflected by the similar disintegration count during the first 10 minutes of recording (Table I). Variations occurring during the first few minutes were probably due to the slightly different time intervals from time of injection until proper geometry was established, and to the fact that only scaler meter counts were recorded (it being impossible to estimate interpolation figures in this continuous study). Similarly, the difference seen in the ninth minute is probably explained by a transient alteration of geometry, as it is due to 1 aberrant observation.

TABLE I

MEAN AND RANGE OF OBSERVATIONS AT EACH OF THE FIRST 10 MINUTES

TREATMENT		TIME IN MINUTES									
		1	2	3	4	5	6	7	8	9	10
I^{131} No NaF	Mean	102	268	383	550	645	768	831	934	1,049	1,062
	Range	64	128	64	128	158	258	384	256	320	384
I^{131} + Intraperitoneal dosage NaF	Mean	76	191	403	524	652	703	818	921	870	1,010
	Range	128	256	320	576	640	704	832	704	640	768

Experiment II.—This phase was accomplished to test the effect that a continuous fluorine intake superimposed on an iodine deficiency would have on thyroid iodine uptake and plasma iodine level.

The study involved 48 rats, divided into 4 groups, each group including subsections of 6 female and 6 male rats. All 4 groups were placed on an iodine-deficient diet, as suggested by Remington,[29] for a period of 35 days, to deplete

the iodine stores of the body. During this time, each group of rats was allowed ad libitum access to demineralized water containing a definite level of fluorine. The control group (Group C) was given no fluoride. Group L received a low increment of 10 ppm of sodium fluoride, Group M received 50 ppm, while Group H received 100 ppm.

At the end of 35 days, and continuing through the ensuing week, blocks of animals, picked equally from each group and subsection, were given an intraperitoneal injection of 1 ml. of saline solution, containing 1 μc of activity as iodine-131. After 24 hours, each rat was anesthetized and exsanguinated via the abdominal aorta. The thyroid glands were excised, weighed, and placed in 3 ml. of 2N sodium hydroxide for digestion.

The collected heparinized blood was centrifuged at 2,500 r.p.m. for a period of 1 hour, after which an aliquot of plasma was placed in a tared 2 ml. vial, weighed, and set aside for eventual deep-well scintillation counting.

Actual counting was accomplished by utilization of a Tracerlab scaler, with a specially modified high-power supply to ensure circuit stability. All gross counts recorded for the standard iodine solution, as well as for the thyroid digest and plasma, were corrected for background, decay, and aliquot factor. Data were compiled on the following: counts per gram of thyroid, per cent of uptake of the thyroid, and counts per gram of plasma (Table II).

TABLE II

		NaF (MG.) CONSUMED (43 DAYS AV.)	TOTAL COUNT	PER CENT UPTAKE	TOTAL COUNT GM. PLASMA
C	Female	0	513,860	60.5	3,009
	Male	0	479,310	56.6	2,514
	Average	0	496,585	58.5	2,761
L	Female	9	454,143	53.8	2,123
	Male	11	501,680	58.5	2,569
	Average	10	477,911	56.1	2,346
M	Female	48	532,092	62.6	2,048
	Male	53	460,584	54.6	2,121
	Average	50.5	496,338	58.6	2,084
H	Female	79	576,102	68.3	1,686
	Male	104	529,551	62.9	2,704
	Average	91.5	552,826	65.6	2,195

In all 4 groups, the disintegration count for the digested thyroid, and for the blood plasma, was of a similar magnitude. It will be noticed in the last column of Table II that the counts per gram of blood plasma of the females exhibit a downward trend with increasing fluorine supplement. Statistical analysis demonstrated that these values did not differ significantly, due to the large individual variation within each group.

In order to secure additional information on the distribution of the iodine-131, 1 female rat from each group was given 250 μc of activity (instead of 1 μc), following which they were fasted and deprived of water for a period of 24 hours. At this time, they were sacrificed, thyroids excised, and fixed with

formalin. Autoradiograms were then made by positioning the resultant tissue sections on Eastman Kodak NTB plates and staining with hematoxylin (Figs. 1 to 5).

Fig. 1.

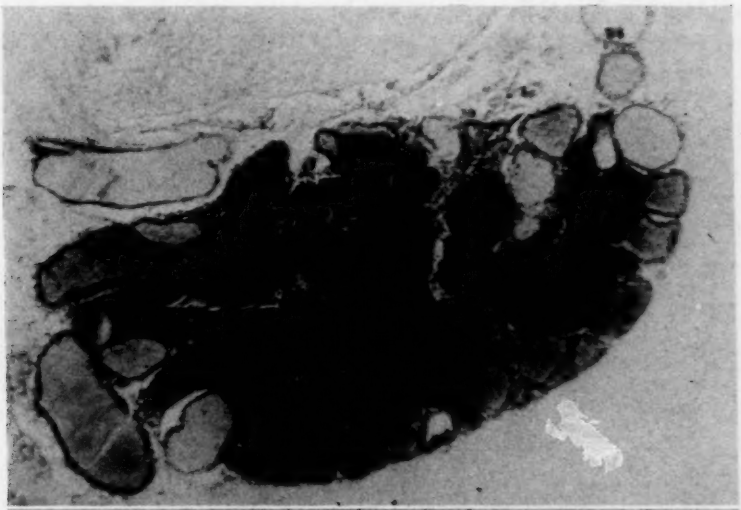


Fig. 2.

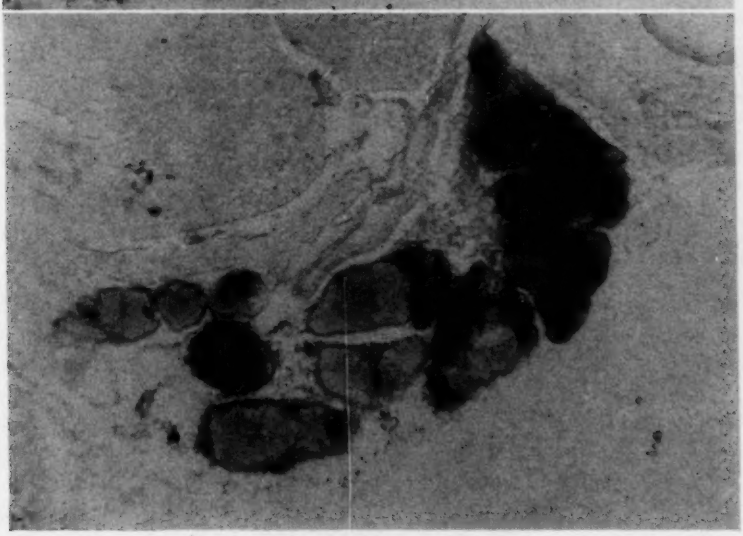


Fig. 1.—Autoradiogram. Control thyroid. Note activity of colloid of follicles, with scattered foci of extreme concentration (×64).

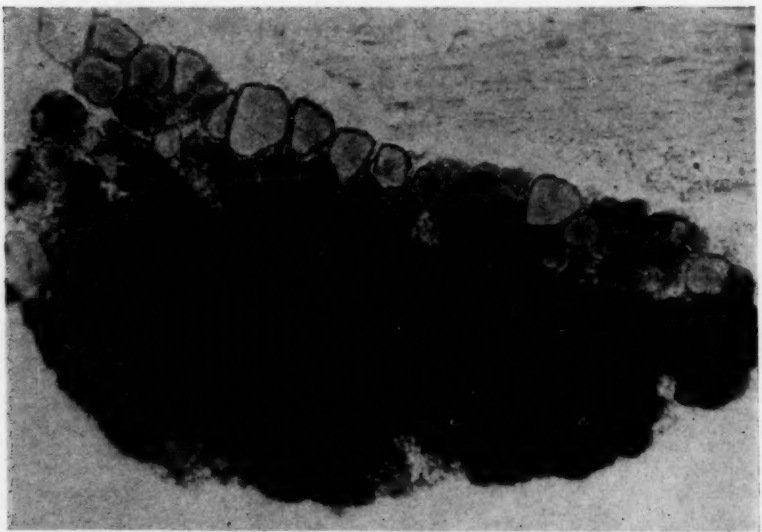
Fig. 2.—Autoradiogram. Thyroid gland from rat on low increment of sodium fluoride (10 ppm) (×64).

DISCUSSION

This experiment has been designed to demonstrate the effect of acute and chronic exposure of fluorine on thyroid iodine metabolism. If the fluorine acted to inhibit the uptake of iodine by the thyroid, then a marked decrease in count

could be expected in the fluorine-treated animals. If such an alteration of glandular physiology was due to a competitive uptake between the fluorine and iodine, then the inhibition would be inversely proportional to the dosage of

Fig. 3.



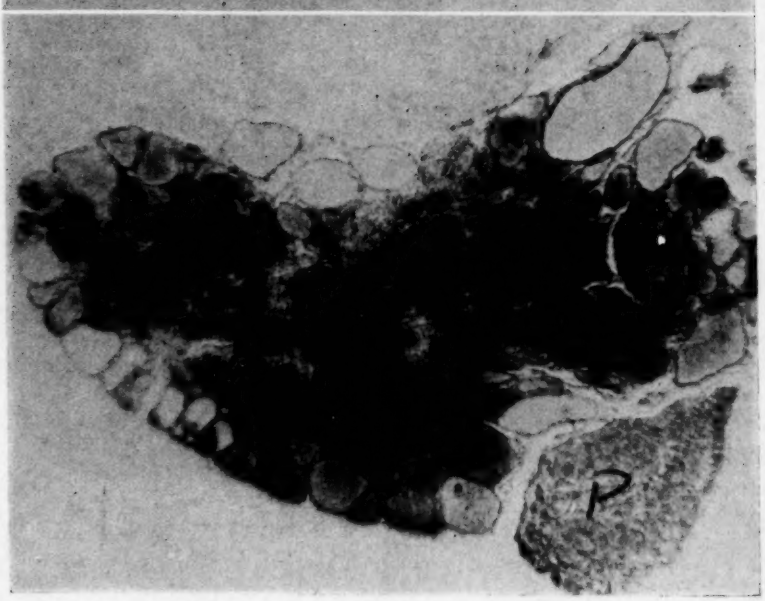


Fig. 4.

Fig. 3.—Autoradiogram. Thyroid gland from rat on medium increment of sodium fluoride (50 ppm) (×64).

Fig. 4.—Autoradiogram of thyroid from rat fed high increment of sodium fluoride (100 ppm). Dispersion and intensity of activity much the same as in Figs. 1 to 3. Note lack of activity in parathyroid gland (*P*) (×64).

sodium fluoride. If, on the other hand, the fluoride caused an enzyme inhibition, the cut-off point in the uptake gradient would be well defined. Autoradiographs would further demonstrate qualitatively, and to some extent quantitatively, whether there was an uptake of iodine-131, and whether this uptake was eventually followed by the normal transfer of activity from the acinar cells to the colloid. Finally, the plasma iodine-131 level should reflect the efficiency of distribution of the element to the blood.

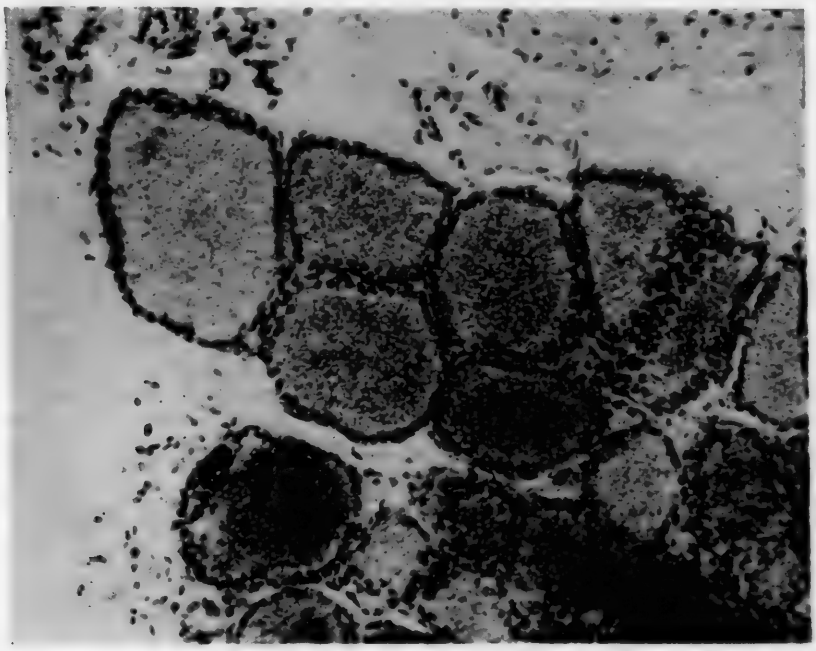


Fig. 5.—Autoradiogram. Thyroid gland from rat on medium increment of sodium fluoride (50 ppm) (×180). Note grain to be seen in colloid, due to iodine-131 activity.

In the cases involving chronic exposure to sodium fluoride, autoradiograms made of the thyroid tissue 24 hours after administration of iodine-131 demonstrated that the iodine activity was concentrated in the colloidal phase, regardless of the level of dietary fluorine intake. This would indicate that the iodine that was taken up by the glands in both the treated and control cases was transported equally well through the acinar cell phase.

Digestion counts likewise demonstrated the same statistical uptake gradient of iodine activity for each group and sex. Also, the similarity of magnitude of the plasma iodine-131 counts indicated that fluorine had no inhibitory effect on the distribution of the iodine content to the blood plasma.

Thus it appears that with either acute dosage of fluorine, or chronic administration of fluorine superimposed on a dietary iodine deficiency, there is no inhibition of uptake of iodine-131, transport of same to the colloid, or eventual release into the vascular system. It must be emphasized, though, that despite

the fact that no significant alteration in thyroid iodine metabolism was noted, the possibility of chemical alteration of the iodothyroglobulin at points other than the iodine bond is a possibility. This might explain the synergistic effects seen by Wilson and De Eds[33] and Phillips, English, and Hart.[24]

SUMMARY

In a study designed to test the possible inhibitory effect of acute and chronic exposure to sodium fluoride on the uptake gradient and metabolism of iodine-131 by rat thyroids, the following facts were elicited:

1. Neither acute nor more chronic dosage of sodium fluoride inhibited the uptake of iodine-131 by the thyroid gland.

2. The transport of the iodine-131 through the acinar cell stage into the colloidal phase, and ultimate distribution to the blood plasma, were of equal magnitude, regardless of level of sodium fluoride used in the study.

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THE EFFECT OF DIFFERENT FLUORIDES ON VASCULAR TONE AND ALTERATION IN THE RATE OF BLOOD FLOW

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THE current interest in the relationship of fluorine to dental caries has accentuated studies related to its metabolic activity in the body.[1] Recently, McClure[2] reported on the fluoride content of various foods and suggested that, while varying widely with the diet ingested, the daily intake of fluorine from typical sources of food alone was about 0.3 mg. It was estimated that the typical daily fluorine intake in areas of fluoridated water consumption was 1.4 to 1.8 mg. To generalized statements concerned with fluorine ingestion, notable exceptions must be made regarding special food substances. For example, sea foods may contain 5 to 25 ppm fluorine, and tea 75 to 100 ppm (about 0.12 mg. fluorine per cup). Milk may contain 0.1 to 0.3 mg. fluorine per liter. Also, the daily fluorine intake varies considerably due to such factors as the natural individual variation in diet consumption, the age of the individuals, fluorine availability in the various nutrients, climate, and the total amount of daily food and water intake.

The concept that fluorine might affect the flow of saliva has been suggested. It was pointed out by McClure[3] that one of the effects of excessive fluorine administration was an acceleration of the flow of saliva. Rathje[4] postulated that the reduction in dental caries produced by fluorine may be mediated through the thyroid gland by increasing salivary flow, and Muhler and Shafer[5] have reported a reduction in the incidence of dental caries in the rat by feeding desiccated thyroid, and an increase when feeding thiouracil.

It was a purpose of our studies to evaluate the action of fluorine in the blood upon the vascular resistance of the body to blood flow, along with any concomitant alterations in the rate of volume blood flow. Since it also has been reported[6, 7] that fluorides vary in their physiologic activity, 4 different fluorides were chosen for these vascular studies. The two most obvious routes for fluorine to affect the dental enamel are by way of the blood stream during the calcification processes, and through the saliva when the tooth has erupted. Some workers feel that saliva volume per se influences the condition of the

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teeth.[8] However, others believe that a concentration of fluorine sufficient to affect the erupted tooth is not excreted by the salivary glands.[9] The production of saliva is itself influenced by the volume blood flow in the environment of the salivary glands; thus, it seemed doubly pertinent to assay quantitatively the effect of fluorine on vascular circulation.

PROCEDURE

Blood flow and blood pressure measurements were made in the femoral arteries of mongrel dogs, 3 to 4 cm. below the inguinal ligament. Six to 9 animals were used for each variable, with each animal serving for multiple assays. These animals weighed from 8 to 18 kilograms, and were anesthetized with pentobarbital sodium, 33 mg. per kilogram, given intravenously. Heparin, 5 mg. per kilogram, was given as an anticoagulant. After the administration of these agents, at least 20 to 30 minutes were allowed to elapse before assays were initiated in order to minimize any effect of these agents on the vascular system.[10]

The rate of volume blood flow was recorded by use of the electromagnetic blood flow meter similar to that as described by Richardson, Denison, and Green,[11] modified from the design of Richardson, Randall, and Hines.[12] This instrument features a linear reproduction of flow transients within 5 per cent error forward and backward, with a negligible drift. Blood pressures were recorded by use of a P-23A Statham strain gauge and a Brush strain analyzer and ink-writing oscillograph. The peripheral vascular tone was calculated in peripheral resistance units (PRU) as described by Green.[13] Room temperatures, rectals, and gastrocnemius muscle temperatures were measured by 3 thermocouples housed in hypodermic needles, as described by Tuttle and Janney.[14] These were attached to a Brown electronic automatic chart-printing recorder.

Four fluoride salts, NaF, SnF_2, KF, and Na_2PO_3F, were assayed. All solutions were administered intravascularly in 0.1 to 0.5 ml. quantities. It was felt that larger quantities might create artifacts due to blood dilution and, thereby, introduce a second variable which might contribute to blood flow response in itself. All concentrations were isotonic in order to eliminate the possibility that anisotonicity might contribute to the response. Responses were compared to control resting blood flows previous to each assay, but in all cases a second series of controls were made using equivalent amounts of NaCl as a precaution that the injection procedure did not influence the measured responses ascribed in the fluorides. Because of the marked response of SnF_2, a separate series was performed with stannous gluconate and stannous chloride to delineate better the effect of the stannous ion. In the assays, 5 to 20 minutes were allowed between fluoride observations, and standardized dosages of epinephrine, mecholyl, and acetylcholine were used to evaluate the reactivity of the vascular bed when this information was needed. The pH

of the solutions was measured by a Leeds and Northrup a.c.-powered pH meter, because of the possibility that deviations in hydrogen ion concentration of sufficient magnitude might alter the flow response.[15]

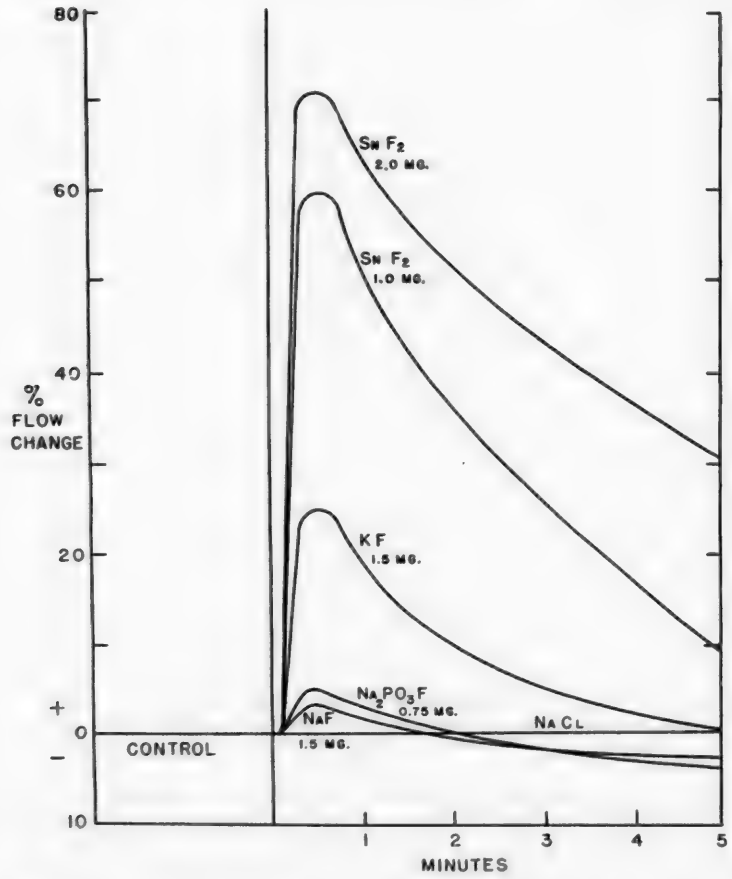

Fig. 1.—Blood flow response to the intravascular administration of different fluorides. The milligram weights refer to the amount of added fluorine. The NaCl added was isotonic in 0.5 ml. portions.

RESULTS

I. *Studies of Acute Reactions of the Vascular System to Fluorine.*—

A-1. *Alterations in the rate of volume blood flow due to fluorides, in the arterial blood:* Since the electromagnetic blood flow meter allows a continuous record of blood flow after a control period, the recording of flow responses was carried out continuously over a 5-minute period after each injection. Five assays of 4 different chemicals containing fluorine were made. These included the following amounts of fluorine in milligrams: Na_2PO_3F, 0.75; NaF, 1.50; KF, 1.50; SnF_2, 1.00; and SnF_2, 2.00.

The first 4 values were selected because they allowed a reasonable variation in fluorine content, while keeping the solutions isotonic. Prior to the experiments, it was not known whether different fluorides would evidence any different action on the vascular system. Early in the studies, however, it became apparent that SnF_2 injected into the blood created a greater vasodilation and greater flow acceleration than the others. Exploratory studies showed that when more than 5 mg. of fluorine as SnF_2 was added to the blood stream, it altered the vascular bed over long periods to the extent that the animal was unsuitable for further quantitative assays. This phenomenon is even somewhat apparent with lesser amounts. For this reason, all administrations were given in random sequence except SnF_2, which was given last.

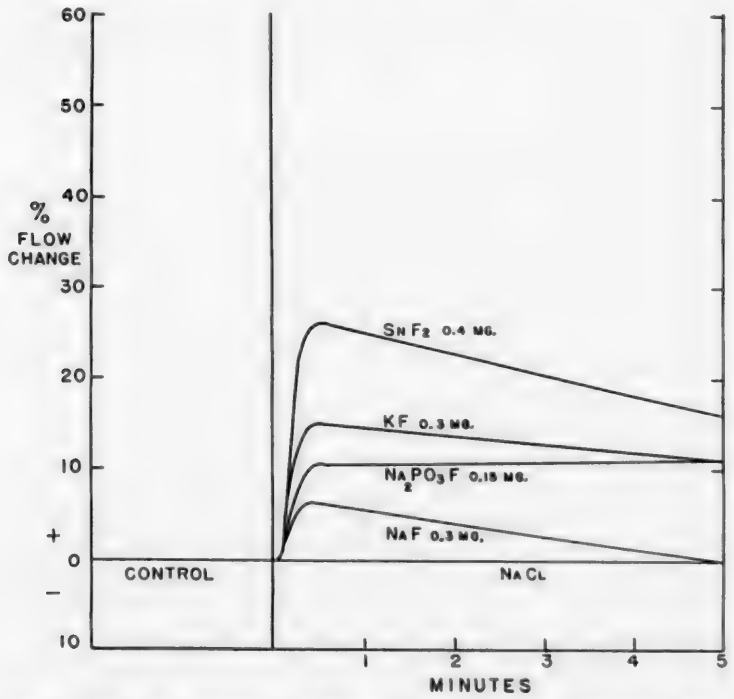

Fig. 2.—Blood flow responses as in Fig. 1, but with smaller amounts of fluorine.

The results of this series of studies are shown in Fig. 1, where it can be seen that SnF_2 was the most responsive fluoride in the production of increased blood flow. When 1 mg. of fluorine as SnF_2 was added to the blood, it increased the blood flow 59 per cent in 20 seconds, but a second addition of twice this amount (2 mg. F) increased the flow only 71 per cent, suggestive of tachyphylaxis. The action of SnF_2 also was more prolonged than the other fluorides. In all cases, however, the flow increases had a greater duration

than observed following 0.1 mg. of acetylcholine or mecholyl (40 to 60 seconds), although the blood flow increases were comparable over a 10- to 20-second period.

It has been reported[16] that the normal content of fluorine in the dog per liter of circulation blood is about 0.21 (0.11 to 0.30) mg., or about 0.21 ppm. Although it was determined that various fluorides increase blood flow, the quantities used were considerably in excess of the reported normal blood values. Therefore, a second series of studies was carried out using smaller quantities in isotonic solution.

A-2. *Alterations in the rate of volume blood flow due to lesser amounts of fluorine in the blood:* This series of experiments followed the same procedure as the first, except that only one concentration of SnF_2 was used. The fluorine content of the individual injections varied from 0.15 to 0.4 mg. Significant flow increases in a prolonged action of the different fluorides also were seen in this series. Fig. 2 shows the relative activity from SnF_2 and NaF, and Table I contains a summary of the values of this and the previous series.

TABLE I

SUMMARY OF FLOW RESPONSE AND VASCULAR RESISTANCE CHANGES AS A RESULT OF FLUORIDE ADMINISTRATION

SOLUTION	F (MG.)	% FLOW INCREASE AFTER 20 SECONDS		% DECREASE PRU AFTER 20 SECONDS	
		TOTAL	PER 0.5 MG.	TOTAL	PER 0.5 MG.
SnF_2	1.0	59		43	
	0.4	27	31.5	22	24.5
KF	1.5	25		28	
	0.3	15	16.7	11	13.8
Na_2PO_3F	0.75	7		9	
	0.15	10	8.3	8	8.5
NaF	1.5	4		6	
	0.3	6	5.7	6	6.0
NaCl	0	0		0	

It was considered of interest that the normal rate of blood flow could be altered by fluorine in these small magnitudes. The rate of volume blood flow increased from 6 to 27 per cent with the different fluorides assayed.

B-1. *Effect of fluorine on the alteration of the vascular tone of small vessels:* The blood pressure and blood flow records were analyzed at intervals before and after fluorides were added, and the vascular resistance was calculated in PRU units as described in the procedure. The changes in vascular resistance due to the different fluorides can be seen in Table I, and in Figs. 3 and 4. In all cases, there was a prolonged decrease in vascular tone comparable in degree to the flow increases. Minor differences in the magnitude and duration of responses were due to slight changes in blood pressures. The ratio of effectiveness was maintained, wherein SnF_2 was most effective, NaF was least effective, and KF and Na_2PO_3F fell between these two, per 0.5 mg. of fluorine.

II. *Studies of Prolonged Reactions in the Vascular System to Progressive Increments of Fluorine.—*

A-1. *Latent effect of fluorine on the rate of volume blood flow and vascular tone:* Progressively increased amounts (0.15 to 9.0 mg. F) of NaF, KF, Na_2PO_3F, and SnF_2 were introduced into the arterial vascular system in random sequence after a control period of blood flow measurement, and the rate of blood flow was measured 5 minutes after each increment of added fluorine.

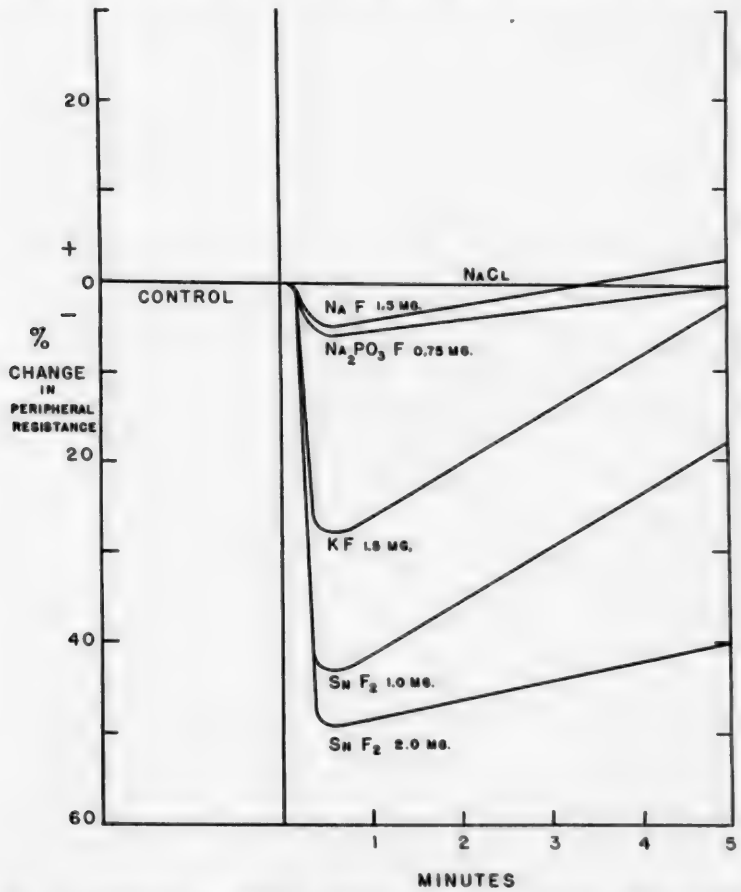

Fig. 3.—Alterations in vascular resistance due to intravascular fluoride administrations, calculated in PRU where PRU = $\frac{\text{mm. Hg pressure}}{\text{ml./min. flow}}$. The ordinate is calibrated as per cent of control values. These values correspond to the flow data in Fig. 1.

Blood pressures were recorded, and the vascular tone calculated for each condition. The results of progressively increased blood-fluorine levels upon blood flow are indicated in Fig. 5. Each position on the curve represents the average blood flow 5 minutes after the fluorine was added. With additions of fluorine up to about 1 mg., acceleration in the blood flow was roughly pro-

portional to the increased levels of fluorine. In the range of 1 to 5 mg. of added fluorine, there was significantly less flow response per milligram of fluorine after 5 minutes. The blood pressure was maintained within narrow limits up to 5 mg. of fluorine, but further addition of fluorine elicited a steady decline in blood pressure, along with more pronounced blood flow acceleration. Fig. 6 shows the concomitant changes in vascular tone. The loss of vascular tone closely followed the increases in blood flow except in the last phase of the studies (5 to 9 mg. F), where the loss of blood pressure somewhat affected the flow response.

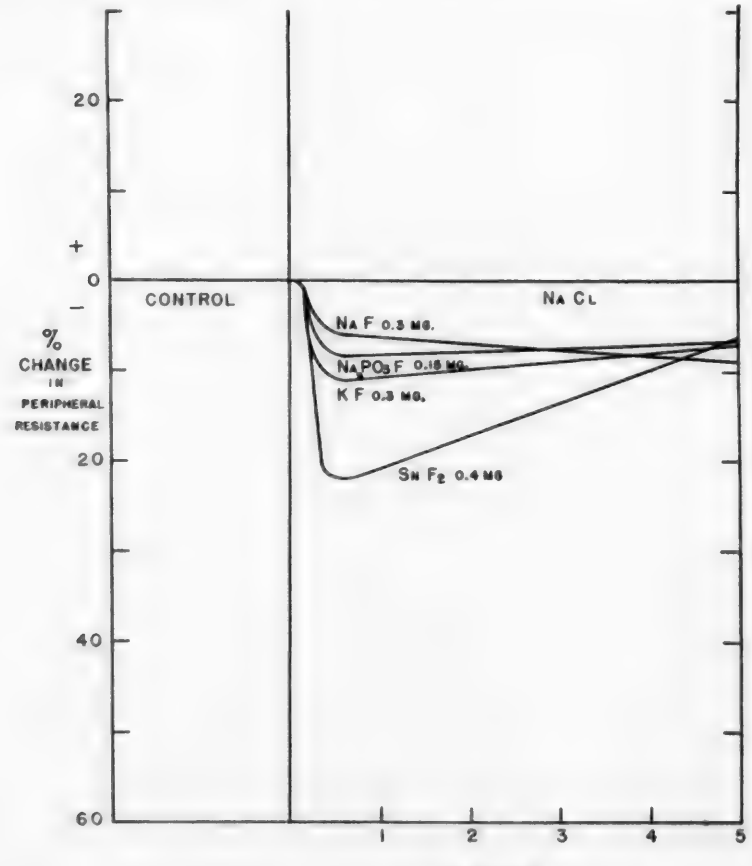

Fig. 4.—Alterations in vascular resistance as in Fig. 3, but with smaller amounts of fluorine as in Fig. 2.

III. *Additional Correlated Studies.*—

A. *Respiration:* During the progressive administration of fluorine up to 9 mg., the respiratory rate increased from a control value of 10.4 per minute to 17.8 respirations per minute.

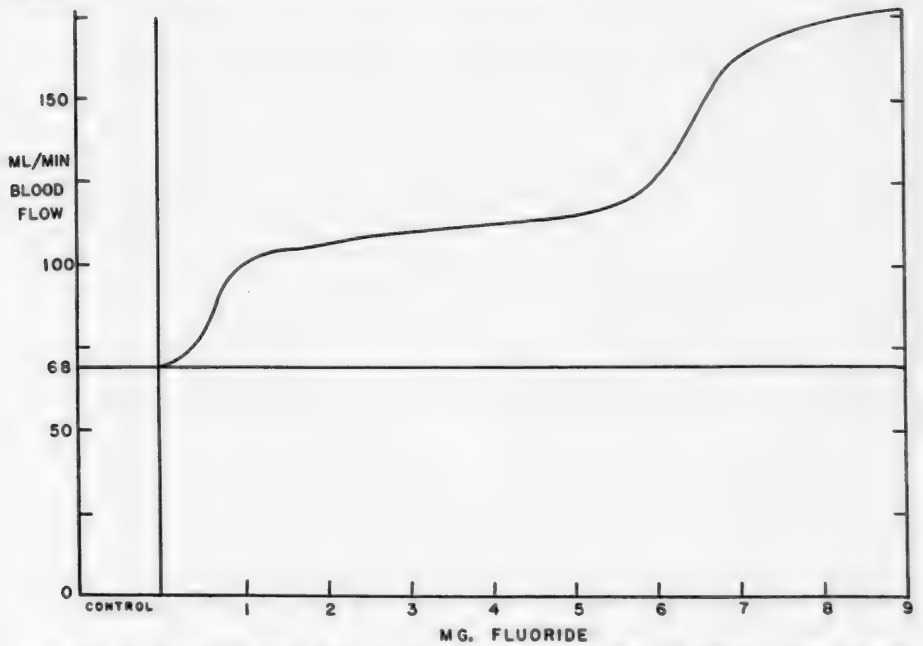

Fig. 5.—A plot of blood flow changes to increased amounts of fluorine. Each position on the curve represents the prolonged elevation of the rate of blood flow 5 minutes after each intravascular administration of fluorine.

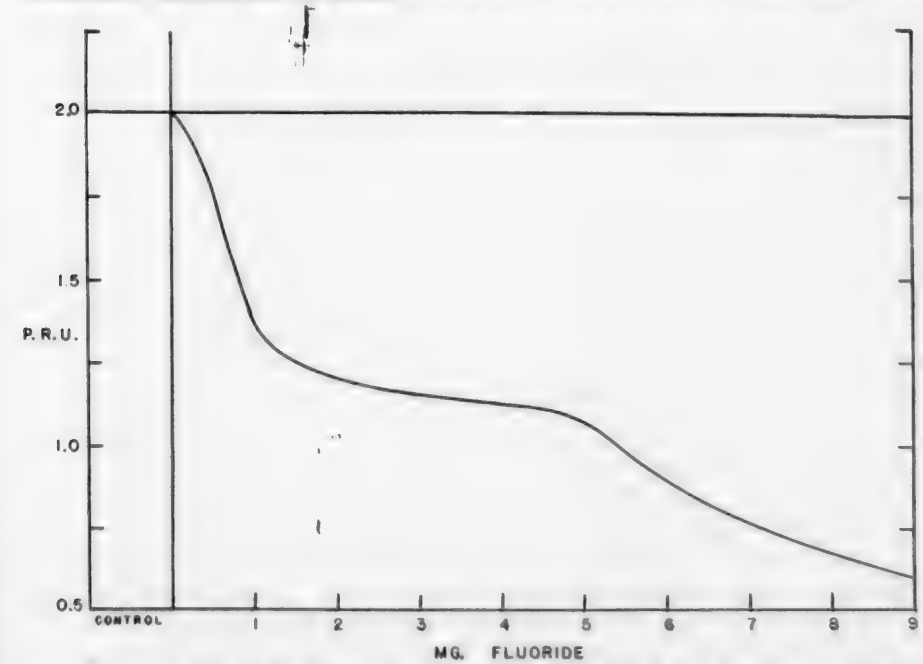

Fig. 6.—A plot of the changes in vascular resistance due to the intravascular addition of progressive amounts of fluorine. Each position on the curve represents the resistance in PRU 5 minutes after each addition of fluorine. The prolonged vasodilator action of fluorine on the vascular bed is apparent.

B. *Muscle temperature:* The gastrocnemius muscle temperature at a 1 cm. depth declined from an initial temperature of 37.7° C. to 34.1° C., but control studies showed that 75 per cent of this drop in temperature was due to the anesthesia.

C. *Blood pressure:* The control mean blood pressure of the animals averaged 123 mm. of mercury. After the injection of 9 mg. of fluorine, the blood pressure decreased to 103 mm. Hg, a decline of 16 per cent. Alterations in heart rate followed no specific pattern, but the pulse pressure diminished with mean arterial pressure, suggesting a diminution in the stroke volume of the heart.

D. *pH measurements:* It has been shown[15] that the introduction of acidic or basic solutions into the blood stream elicits alteration in blood flow in the hydrogen ion concentration which is appreciably different from normal blood values. This is more true of buffered solutions than unbuffered, and the response is characteristically transient. The pH values of the agents used were NaF, 6.9; KF, 6.8; Na_2PO_3F, 6.4; and SnF_2, 3.2. Unbuffered solutions at the pH values of the first 3 fluorides give a negligible vascular response, but a solution at pH 3.2 in 0.1 to 0.5 ml. quantities might account for a 5 to 10 per cent increase in blood flow, but pH per se does not account for the high activity of SnF_2 over the other fluorides studied.

E. *Action of Sn^{++} on the vascular system:* The introduction of 1 mg. of tin as stannous gluconate (pH 5.6) elicited a flow increase of 20 per cent. $SnCl_2$ (pH 2.3), containing 0.78 mg. of Sn, was assayed and was found to elicit an 18 per cent increase in blood flow, demonstrating that the tin component of SnF_2 contributed significantly to the high activity of SnF_2 on the vascular system. Both responses were transient, lasting 40 seconds or less.

COMMENT

These data offer a basis for considerable speculation and lead to a number of further questions. First of all, they offer a mechanism by which fluorine may stimulate the flow of saliva. Vasodilation is one such mechanism, it is agreed, that will enhance the flow of saliva. This may mean that one of the beneficial effects of fluorine in lowering the incidence of dental caries is by way of increasing salivary flow.

These findings pose an interesting question as to what they represent in terms of the dietary-ingested fluorides. An evaluation of this question involves two major factors, the ratio of fluorine absorbed to that ingested, and the amount of circulating blood in the human body compared to that in the animals used for these assays. It is a safe assumption that an adult human body contains 5 to 6 times as much blood content as the animals studied (average, 1 liter), but a child of 30 pounds should possess a blood content comparable to the animals employed. Experiments measuring the vascular alterations from fluorine administered orally are in progress.

Another question of interest is how these findings may be evaluated in terms of fluctuations in the human diet relative to fluorine intake. It is feasible that 0.12 mg. of fluorine in a cup of tea, or 0.05 to 0.15 mg. in a pint of milk, may have a mild effect on the cardiovascular system were it in such a form as to be readily metabolized. Chemically, sodium fluoride in the communal water supply is readily available. It is recommended that about 1.0 mg. of fluorine per day be ingested from such communal water supplies alone, in order to provide an optimal concentration for dental health. In light of the data presented in these experiments, it would seem evident that salivary flow might be increased by such a procedure. Further studies along these lines are needed in order to ascertain if human salivary flow is greater in areas with naturally occurring fluorides as opposed to areas with very low fluoride levels, as well as accurate analysis of the relationship between salivary volume and dental caries initiation.

SUMMARY

1. Fluorine added to the blood acts as a vasodilator. Flow increases may be elicited by 0.15 mg. or more fluorine, and amounts in excess of 5 mg. result in a decline in blood pressure.

2. The cation of a fluoride compound influences its activity. The order of activity of 4 fluorides studied was SnF_2, KF, Na_2PO_3F, and NaF.

3. The action of fluorine is longer than most vasodilators, and concomitant studies of respiration and muscle temperatures suggest that it may act to depress body cell metabolism, in the higher concentrations studied.

4. It is believed that these findings suggest a mechanism by which fluorine may stimulate the flow of saliva which has been reported but not well explained.

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THE EFFECT OF FLUORIDES ON THE ORGANIC AND INORGANIC COMPONENTS OF INTACT DENTIN

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INTRODUCTION

THE decrease in solubility of the inorganic components of dentin and enamel in decalcifying acids is a method by which fluorides are thought to prevent the onset or to decrease the progress of dental caries.[1] The acid solubility-decreasing effect of fluorides has been investigated most often,[2-11] but other ions, such as lead,[4, 7, 9, 10] silver,[7, 9, 12] copper,[7, 8, 10] barium,[9] tin,[7] thorium,[7] and uranyl[7] in various chemical combinations, have also been studied under somewhat comparable conditions. The decalcifying acids most often used to test the solubility of tooth tissues in vitro are acetic[2, 4, 8, 10] and lactic acid.[5, 9, 12] Lactobacillus cultures have also been used to determine the solubility of such tissues.[13] More recently, the effect of 147 compounds, comprised of 57 elements, was tested on the acid solubility of powdered enamel.[10] From that study 27 compounds were reported to depress the solubility of the powdered enamel in vitro more than fluorides, and several fluorides were found to be more effective than sodium fluoride. Nevertheless, very few ions other than fluorine have been of practical value in preventing dental caries.

In spite of the widespread use of fluorides as caries-preventive agents, few studies have been made of their specific action upon both the organic and the inorganic components of intact enamel or dentin. Furthermore, in such studies very little consideration has been given to the action of different types of acids upon enamel or dentin treated with different fluorides. Since the action of fluorides on either intact or pulverized dentin has been investigated even less than that of enamel, and since a diazotization process for determining the accessibility of tyrosine and histidine residues of the organic matrix of intact or decalcified dentin has been developed,[14] it was felt that more detailed information concerning the action of various fluorides on intact, calcified tissues of teeth could be obtained by studying their effect on the accessibility of both the inorganic and organic components of dentin subjected to acid decalcification. The present paper describes the general effect of 5 fluorides on the accessibility of the inorganic and organic components of human dentin exposed to 6 organic and 2 inorganic acids.

METHODS

The sections of dentin utilized in this study were obtained from extracted human teeth, selected at random, and which so far as could be ascertained had

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not been exposed to fluorides during their development. After thoroughly cleaning the teeth with brush and vulcanite bur, 8 sections of dentin, usually weighing less than 100 mg., were cut from each tooth with a safe-side disc. Pulp and cementum were removed from the sections, after which they were dried to a constant weight in a vacuum desiccator at room temperature. The intact sections of dentin prepared in this manner were then exposed to 5 ml. of distilled water or to 2 per cent solutions of sodium fluoride, calcium fluoride, lead fluoride, potassium monofluorophosphate, or calcium monofluorophosphate for the desired time. After exposure to the test solutions, the sections were thoroughly washed in distilled water and dried to a constant weight in vacuo at room temperature in preparation for their exposure to decalcifying acids. The decalcifying solutions, composed of 1 per cent peptone in phosphate buffer, were adjusted to pH 3.0, respectively, with lactic, acetic, butyric, citric, succinic, formic, hydrochloric, or nitric acid. Each section of dentin from each tooth was decalcified in 10 ml. of each acid solution for the specified time. Thus sections from each tooth were decalcified with a different acid for the desired time. At the end of the decalcification periods, the acids were removed rapidly and their pH's checked, after which the sections were neutralized with a saturated bicarbonate solution, washed thoroughly in distilled water, and dried to a constant weight in vacuo at room temperature. The weight loss of the untreated and treated sections of dentin was considered to indicate the solubility of their inorganic components in the decalcifying acids.

After determining the weight loss of each dentinal section, the accessibility of their tyrosine and histidine residues was determined by a diazotization process developed by Burnett and Scherp.[14] This method and its application have been described elsewhere[14, 15] and will not be repeated in detail. The method consists of measuring colorimetrically the amount of reddish diazoprotein formed by the specific coupling of a diazonium salt to accessible tyrosine and histidine residues in the protein chain. Such proteolytic enzymes as pepsin or carboxypeptidase will not act upon protein unless such accessible amino acids are adjacent to the dipeptide linkages of the amino acid chain.[16] When the diazotization process was applied to dentinal protein under the conditions described, intact dentin reacted only slightly, but a relatively constant proportion of azoprotein was formed by acid decalcified dentin. Alteration of dentinal protein resulted in the formation of consistently different amounts of azoprotein per unit weight of tissue. Also, partially decalcified dentin formed a relatively constant amount of azoprotein in proportion to the degree of decalcification. Since deviations in the amount of azoprotein formed indicated alteration in protein, the accessibility of the protein of the control and fluoride-treated dentin exposed to 6 organic and 2 inorganic acids was determined by the procedures described previously.

RESULTS

In the first experiment the effect of the treatment of intact dentinal sections with distilled water, sodium fluoride, calcium fluoride, lead fluoride, potassium monofluorophosphate, and calcium monofluorophosphates on the accessibility of

both their inorganic and organic components when exposed to organic and inorganic acids was determined. Eight sections of dentin from each of 24 teeth were divided into 6 groups. The sections from 1 group were treated with the distilled water, while the other 5 groups were treated with 2 per cent solutions of the various fluorides for 144 hours. After treatment, each section from each tooth was decalcified separately in a lactic, acetic, butyric, citric, succinic, formic, hydrochloric, or nitric acid solution at pH 3.0 for 24 hours at 37° C. The mean weight loss and accessibility of the organic matrix for each tooth are compiled in Table I, together with a similar summary for each group of teeth treated with the various fluoride solutions. The change in weight of the dentin sections of each tooth during treatment with water and aqueous solutions of fluorides is also summarized. Only the sections treated with lead fluoride increased in weight; all others lost weight. The means and their standard errors for the accessibility of the inorganic and organic components of the dentin for the individual teeth were calculated on the basis of the individual sections, while those for the various groups of dentin were calculated on the basis of individual teeth rather than on the basis of individual sections. The latter calculation was made to show the variation between teeth rather than between individual dentinal sections, for each section had been exposed to a different acid.

The results indicated that, on the average, sodium fluoride was most effective in decreasing the solubility of the inorganic portion of dentin in the 8 acids, followed in decreasing order by lead fluoride, potassium monofluorophosphate, calcium monofluorophosphate, and finally calcium fluoride. The standard errors for mean weight loss of the sections of dentin from the individual teeth of each group indicated considerable variation in weight loss of such sections. This variation in the weight loss between the individual sections of dentin from the same tooth indicated that the various acids decalcified the sections at different rates even though the acids were all at the same pH. The standard errors for the weight loss of the individual teeth were much less than the standard error for the sections of the individual teeth, since the individual sections for each tooth within a group were subjected to the same series of acids. When the data for the weight loss of the dentin sections were analyzed statistically, it was found that the critical ratios (difference between the means divided by its standard error) between teeth within each group were less than 2 in each case.

The accessibility of the organic dentinal matrices of the dentin sections was compiled in a manner similar to that of the weight loss of the same sections. The amount of variability in the accessibility of the dentinal organic matrix of the individual teeth was greater than the variability of their weight loss. On the other hand, less difference existed between the accessibilities of the organic dentinal matrices of the various groups of dentin than between the weight losses of the same groups. An apparent difference existed between the accessibilities of the organic dentinal matrices of different groups of dentin, with control sections being most accessible, followed in increasing order by sections treated with calcium monofluorophosphate, calcium fluoride, sodium fluoride, potassium monofluorophosphate, and lead fluoride. Statistical analysis

TABLE I

THE ACCESSIBILITY OF DENTIN TREATED WITH FLUORIDES UPON EXPOSURE TO ORGANIC AND INORGANIC ACIDS

TYPE OF TOOTH*	TOOTH NUMBER	NUMBER OF SECTIONS	MEAN WEIGHT OF SECTIONS (GRAMS)	PER CENT WEIGHT CHANGE BY TREATMENT†	PER CENT WEIGHT LOSS†	ACCESSIBILITY OF THE ORGANIC DENTINAL MATRIX†
Control	1	8	0.0358	−2.2 ± 0.2	29.2 ± 6.6	49.4 ± 14.2
	2	8	0.0417	−1.7 ± 0.2	25.6 ± 3.4	76.5 ± 29.6
	3	8	0.0383	−0.4 ± 0.2	25.1 ± 4.6	71.1 ± 28.3
	4	8	0.0397	−1.4 ± 0.3	29.7 ± 7.6	55.7 ± 16.4
					27.4 ± 1.2	63.1 ± 6.3
NaF_2-treated	5	8	0.0357	−0.2 ± 0.3	8.5 ± 6.5	68.6 ± 9.8
	6	8	0.0406	−1.1 ± 0.3	9.7 ± 2.0	54.8 ± 5.0
	7	8	0.0382	−1.4 ± 0.6	9.9 ± 1.6	44.1 ± 4.7
	8	8	0.0468	−1.2 ± 0.1	9.7 ± 1.8	45.7 ± 3.7
					9.4 ± 0.3	53.3 ± 5.6
CaF_2-treated	9	8	0.0455	−1.7 ± 0.2	20.8 ± 3.0	40.2 ± 2.7
	10	8	0.0294	−2.1 ± 0.3	24.9 ± 4.7	40.5 ± 3.4
	11	8	0.0348	−2.8 ± 0.3	23.1 ± 4.2	39.7 ± 4.2
	12	8	0.0191	−2.2 ± 0.5	29.7 ± 5.6	47.8 ± 6.9
					24.6 ± 1.7	42.0 ± 1.9
PbF_2-treated	13	8	0.0309	+0.3 ± 0.2	14.6 ± 2.9	53.3 ± 10.5
	14	8	0.0444	+0.6 ± 0.3	11.2 ± 3.5	62.0 ± 16.8
	15	8	0.0369	+1.3 ± 0.3	9.4 ± 2.0	74.0 ± 17.1
	16	8	0.0246	+1.6 ± 0.4	12.8 ± 2.8	60.3 ± 8.2
					12.0 ± 1.1	62.4 ± 4.3
K_2PO_3F-treated	17	8	0.0295	−1.7 ± 0.2	14.0 ± 1.8	62.4 ± 4.8
	18	8	0.0355	−1.9 ± 0.3	14.1 ± 3.0	52.6 ± 2.1
	19	8	0.0430	−0.9 ± 0.2	14.1 ± 4.2	60.0 ± 2.1
	20	8	0.0389	−0.7 ± 0.4	12.0 ± 2.3	58.5 ± 6.2
					13.6 ± 0.5	58.3 ± 2.1
$CaPO_3F \cdot 2H_2O$-treated	21	8	0.0447	−3.4 ± 0.5	16.6 ± 2.6	42.4 ± 3.2
	22	8	0.0370	−3.3 ± 0.5	17.8 ± 2.9	37.5 ± 3.9
	23	8	0.0367	−4.1 ± 0.9	18.1 ± 2.6	37.9 ± 3.0
	24	8	0.0360		21.5 ± 4.7	37.8 ± 4.3
				−4.1 ± 1.0		
					18.5 ± 1.05	38.9 ± 1.1

*Period of exposure to distilled water and 2 per cent fluoride solutions was 144 hours at 37° C.; one section from each tooth was exposed to lactic, acetic, butyric, citric, succinic, formic, hydrochloric, and nitric acid at pH 3.0 for 24 hours at 37° C.

†Mean and its standard error.

Critical Ratios for Teeth Within a Group

% Weight Loss		Accessibility of the Organic Dentinal Matrix	
Control	All less than 2	Control	All less than 2
NaF_2		NaF_2	Less than 2 except for Teeth 5 versus 7 and Teeth 5 versus 8
CaF_2		CaF_2	All less than 2
PbF_2		PbF_2	All less than 2
K_2PO_3F		K_2PO_3F	All less than 2 except for Teeth 18 versus 19
$CaPO_3F \cdot 2H_2O$		$CaPO_3F \cdot 2H_2O$	All less than 2

Critical Ratios Between Different Groups of Teeth

Critical ratios between percentage weight losses were *greater* than 2 for all groups of dentin except for control versus CaF_2, control versus K_2PO_3F, and PbF_2 versus K_2PO_3F.

Critical ratios between accessibilities of the organic dentinal matrices were *less* than 2 for all groups of dentin except control versus $CaPO_3F \cdot 2H_2O$, control versus CaF_2, PbF_2 versus $CaPO_3F \cdot 2H_2O$, and K_2PO_3F versus $CaPO_3F \cdot 2H_2O$.

of the data for the accessibility of the organic dentinal matrices revealed that the critical ratios between accessibilities of the matrices within the various groups of teeth were less than 2 for the sections of control teeth, and for calcium fluoride-, lead fluoride-, and calcium monofluorophosphate-treated teeth. The critical ratios between the accessibilities of the organic dentinal matrices of the sections of dentin were more than 2 in the case of Teeth 5 versus 7 and 5 versus 8 in the sodium fluoride-treated group and in the case of Teeth 18 versus 19 in the potassium monofluorophosphate-treated group. The critical ratios between the mean accessibilities of the different groups of dentin were less than 2 in 11 of 15 possible comparisons.

In the next series of 8 experiments, data were obtained for the accessibility of the organic and inorganic components of intact dentin when such tissue, treated with water and various fluorides for 144 hours, was exposed to individual acids for 24 hours. In each experiment the 4 sections subjected to each type of treatment were from different teeth rather than all being from the same tooth, as in the first experiment.

The action of lactic acid upon the accessibility of the inorganic and organic portions of treated and untreated groups of dentin is shown in Table II. Control dentin sections lost the most weight when exposed to lactic acid, followed in decreasing order of solubility by the groups of sections exposed to calcium fluoride, calcium monofluorophosphate, potassium monofluorophosphate, lead fluoride, and sodium fluoride. Critical ratios between weight losses effected by the action of lactic acid on control and fluorine-treated dentin were greater than 2 in 12 of 15 possible comparisons.

TABLE II

THE ACTION OF LACTIC ACID ON DENTIN TREATED WITH FLUORIDES

TYPE OF TREATMENT*	NUMBER OF SECTIONS	MEAN WEIGHT OF SECTIONS (GRAMS)	PER CENT WEIGHT CHANGE BY TREATMENT†	PER CENT WEIGHT LOSS†	ACCESSIBILITY OF THE ORGANIC DENTINAL MATRIX†
Control	4	0.0368	−1.7 ± 0.5	54.7 ± 6.6	10.1 ± 2.0
NaF	4	0.0424	+0.2 ± 0.9	15.8 ± 1.8	45.3 ± 1.9
CaF_2	4	0.0390	−1.3 ± 0.1	41.6 ± 3.7	31.3 ± 1.3
PbF_2	4	0.0335	+0.9 ± 0.5	23.7 ± 3.4	37.1 ± 2.2
K_2PO_3F	4	0.0356	−1.2 ± 0.5	25.2 ± 3.7	47.3 ± 3.0
$CaPO_3F{\cdot}2H_2O$	4	0.0453	−2.4 ± 0.4	30.2 ± 1.1	27.0 ± 1.7

*Controls treated with distilled water for 144 hours at 37° C.; other sections treated with an unadjusted 2 per cent solution of fluorides for 144 hours at 37° C.; all sections were exposed to acid solutions at pH 3.0 for 24 hours at 37° C.

†Mean and its standard error.

Critical ratios between weight losses of control and fluorine-treated dentin effected by lactic acid were *greater* than 2 except for control versus CaF_2, PbF_2 versus K_2PO_3F, K_2PO_3F versus $CaPO_3F{\cdot}2H_2O$.

Critical ratios between the accessibilities of the organic matrices of control and fluorine-treated dentin effected by lactic acid were *greater* than 2 except for NaF versus K_2PO_3F.

The accessibility of the organic dentinal matrices of the sections decalcified by lactic acid was least in the control group, followed in increasing order by the sections of dentin of the groups treated with calcium monofluorophosphate, calcium fluoride, lead fluoride, sodium fluoride, and potassium monofluorophosphate. Critical ratios between the accessibilities of the organic matrices of control and fluorine-treated dentin effected by lactic acid were greater than 2 in 14 of 15 possible comparisons.

The effect of acetic acid on the accessibility of the organic and inorganic portions of control and fluorine-treated dentin sections is summarized in Table III. The dentin sections were treated as in the previous experiment except that they were decalcified in acetic acid. The weight lost by the control sections was greater than that lost by any of the fluoride-treated sections, and the order of decreasing solubility of the treated groups of dentin was as follows: calcium fluoride, potassium monofluorophosphate, calcium monofluorophosphate, sodium fluoride, and lead fluoride. In the presence of acetic acid, treatment with calcium fluoride decreased the solubility of dentin least, but the solubility-depressing effects of the other fluorides were quite similar, with dentin exposed to lead fluoride being least soluble but not significantly less than that treated with several other fluorides. The critical ratios between weight losses of control and fluorine-treated dentin were greater than 2 in 9 of 15 possible comparisons.

TABLE III

THE ACTION OF ACETIC ACID ON DENTIN TREATED WITH FLUORIDES

TYPE OF TREATMENT*	NUMBER OF SECTIONS	MEAN WEIGHT OF SECTIONS (GRAMS)	PER CENT WEIGHT CHANGE BY TREATMENT†	PER CENT WEIGHT LOSS†	ACCESSIBILITY OF THE ORGANIC DENTINAL MATRIX†
Control	4	0.0415	−1.7 ± 0.6	38.6 ± 3.4	12.7 ± 3.8
NaF	4	0.0468	−1.3 ± 0.3	12.3 ± 2.2	42.6 ± 5.7
CaF_2	4	0.0404	−1.7 ± 0.5	23.1 ± 3.7	36.9 ± 1.9
PbF_2	4	0.0334	+0.4 ± 0.2	11.2 ± 0.7	51.5 ± 4.6
K_2PO_3F	4	0.0299	−1.4 ± 0.3	14.5 ± 2.1	56.8 ± 2.7
$CaPO_3F{\cdot}2H_2O$	4	0.0507	−2.6 ± 0.3	14.3 ± 1.8	36.6 ± 2.9

*Controls treated with distilled water for 144 hours at 37° C.; other sections treated with an unadjusted 2 per cent solution of fluorides for 144 hours at 37° C.; all sections were exposed to acid solutions for 24 hours at 37° C.

†Mean and its standard error.

Critical ratios between weight losses of control and fluorine-treated dentin effected by acetic acids were *greater* than 2 except for NaF versus PbF_2, NaF versus K_2PO_3F, NaF versus $CaPO_3F{\cdot}2H_2O$, PbF_2 versus K_2PO_3F, PbF_2 versus $CaPO_3F{\cdot}2H_2O$, and K_2PO_3F versus $CaPO_3F{\cdot}2H_2O$.

Critical ratios between the accessibilities of the organic matrices of the control and fluorine-treated dentin effected by acetic acid were *greater* than 2 except for NaF versus CaF_2, NaF versus PbF_2, NaF versus $CaPO_3F{\cdot}2H_2O$, CaF_2 versus $CaPO_3F{\cdot}2H_2O$, and PbF_2 versus K_2PO_3F.

The accessibility of the organic dentinal matrices of control and treated dentin exposed to acetic acid was least in the control sections and greatest in the groups of dentin treated with fluorides. In fact, the accessibility of the organic matrices of the groups of dentin treated with various fluorides ranged from 3 to 4 times that of the control sections. The order of decreasing accessibility of the organic matrices of dentin exposed to acetic acid was as follows: potassium monofluorophosphate, lead fluoride, sodium fluoride, calcium fluoride, calcium monofluorophosphate, and control. The critical ratios between the accessibilities of the various groups of dentin were greater than 2 in 10 of 15 possible comparisons.

The action of butyric acid upon the accessibility of the organic and inorganic components of control and treated dentin is summarized in Table IV. The dentin sections were treated as in the previous experiment except that they were decalcified in butyric acid. The sections treated only with water lost the most weight in the acid solution. Those treated with calcium fluoride and

TABLE IV

THE ACTION OF BUTYRIC ACID ON DENTIN TREATED WITH FLUORIDES

TYPE OF TREATMENT*	NUMBER OF SECTIONS	MEAN WEIGHT OF SECTIONS (GRAMS)	PER CENT WEIGHT CHANGE BY TREATMENT†	PER CENT WEIGHT LOSS†	ACCESSIBILITY OF THE ORGANIC DENTINAL MATRIX†
Control	4	0.0326	−1.0 ± 0.6	18.0 ± 1.7	96.1 ± 9.4
NaF	4	0.0381	−1.2 ± 0.4	4.0 ± 0.4	64.6 ± 14.6
CaF_2	4	0.0243	−3.1 ± 0.7	10.1 ± 0.9	61.9 ± 10.4
PbF_2	4	0.0315	+0.4 ± 0.4	2.7 ± 0.4	104.0 ± 18.7
K_2PO_3F	4	0.0384	−1.8 ± 0.1	7.2 ± 0.5	58.4 ± 6.2
$CaPO_3F{\cdot}2H_2O$	4	0.0389	−4.6 ± 1.1	10.0 ± 1.7	44.7 ± 7.4

*Controls treated with distilled water for 144 hours at 37° C.; other sections treated with an unadjusted 2 per cent solution of fluorides for 144 hours at 37° C.; all sections were exposed to acid solutions for 24 hours at 37° C.

†Mean and its standard error.

Critical ratios between weight losses of the control and fluorine-treated dentin effected by butyric acid were *greater* than 2 except for CaF_2 versus $CaPO_3F{\cdot}2H_2O$.

Critical ratios between the accessibilities of the organic matrices of control and fluorine-treated dentin effected by butyric acid were *greater* than 2 except for control versus NaF, NaF versus CaF_2, NaF versus K_2PO_3F, NaF versus $CaPO_3F{\cdot}2H_2O$, CaF_2 versus PbF_2, CaF_2 versus K_2PO_3F, CaF_2 versus $CaPO_3F{\cdot}2H_2O$, PbF_2 versus K_2PO_3F, and K_2PO_3F versus $CaPO_3F{\cdot}2H_2O$.

calcium monofluorophosphate lost approximately the same amount of weight, followed in decreasing order of solubility by the sections treated with potassium monofluorophosphate, sodium fluoride, and lead fluoride. Statistical analysis of the percentage weight loss caused by butyric acid showed that the critical ratios between the various groups of dentin were greater than 2 in 14 of 15 possible comparisons.

The accessibility of the organic dentinal matrices of the groups of dentin exposed to butyric acid was fairly high, with the sections treated with lead fluoride being most accessible, followed in decreasing order by control sections and sections treated with sodium fluoride, calcium fluoride, potassium monofluorophosphate, and calcium monofluorophosphate. The critical ratios between the accessibilities of the various groups of dentin were less than 2 in 9 of 15 possible comparisons.

The effect of citric acid on control and fluoride-treated dentin is summarized in Table V. The dentin sections were treated as in the previous experiment

TABLE V

THE ACTION OF CITRIC ACID ON DENTIN TREATED WITH FLUORIDES

TYPE OF TREATMENT*	NUMBER OF SECTIONS	MEAN WEIGHT OF SECTIONS (GRAMS)	PER CENT WEIGHT CHANGE BY TREATMENT†	PER CENT WEIGHT LOSS†	ACCESSIBILITY OF THE ORGANIC DENTINAL MATRIX†
Control	4	0.0481	−1.2 ± 0.7	21.0 ± 2.0	34.3 ± 5.9
NaF	4	0.0371	−1.1 ± 0.7	7.9 ± 1.1	47.3 ± 9.1
CaF_2	4	0.0374	−2.0 ± 0.4	32.0 ± 7.7	39.2 ± 1.9
PbF_2	4	0.0438	+0.4 ± 0.2	15.6 ± 4.1	50.5 ± 12.0
K_2PO_3F	4	0.0341	−1.1 ± 0.9	16.2 ± 5.6	66.8 ± 5.1
$CaPO_3F{\cdot}2H_2O$	4	0.0340	−2.7 ± 0.9	20.6 ± 2.4	37.0 ± 2.7

*Controls treated with distilled water for 144 hours at 37° C.; other sections treated with an unadjusted 2 per cent solution of fluorides for 144 hours at 37° C.; all sections were exposed to acid solutions for 24 hours at 37° C.

†Mean and its standard error.

Critical ratios between weight losses of control and fluorine-treated dentin effected by citric acid were *less* than 2 except for control versus NaF.

Critical ratios between the accessibilities of the organic matrices of the control and fluorine-treated dentin effected by citric acid were *less* than 2 except for control versus K_2PO_3F, CaF_2 versus K_2PO_3F and K_2PO_3F versus $CaPO_3F{\cdot}2H_2O$.

except that they were decalcified in citric acid. In the acid solution the sections treated with calcium fluoride lost the most weight, followed in decreasing order of solubility by control sections, and calcium monofluorophosphate-, potassium monofluorophosphate-, lead fluoride-, and sodium fluoride-treated sections. The difference in solubility of the dentin in citric acid is more apparent than real, however, because the critical ratios between weight losses of control and fluorine-treated dentin were less than 2 in 14 of 15 possible comparisons.

The accessibility of the organic matrices of the control and fluorine-treated dentin fitted into no particular pattern. The organic matrix of control dentin was least accessible followed in increasing order by groups of dentin treated with calcium monofluorophosphate, calcium fluoride, sodium fluoride, lead fluoride, and potassium monofluorophosphate. The critical ratios for the accessibility of the control and fluorine-treated dentin exposed to citric acid were less than 2 in 12 of 15 possible comparisons.

The accessibility of the inorganic and organic components of treated and untreated dentin in succinic acid is summarized in Table VI. The dentin

TABLE VI

THE ACTION OF SUCCINIC ACID UPON DENTIN TREATED WITH FLUORIDES

TYPE OF TREATMENT*	NUMBER OF SECTIONS	MEAN WEIGHT OF SECTIONS (GRAMS)	PER CENT WEIGHT CHANGE BY TREATMENT†	PER CENT WEIGHT LOSS†	ACCESSIBILITY OF THE ORGANIC DENTINAL MATRIX†
Control	4	0.0444	−1.5 ± 0.4	17.7 ± 1.90	49.9 ± 11.4
NaF	4	0.0356	−1.6 ± 0.5	6.3 ± 0.41	62.7 ± 12.6
CaF_2	4	0.0393	−2.0 ± 0.2	16.4 ± 1.90	51.3 ± 3.8
PbF_2	4	0.0225	+1.3 ± 0.6	4.9 ± 0.78	98.6 ± 19.1
K_2PO_3F	4	0.0431	−1.2 ± 0.2	7.8 ± 1.00	60.9 ± 3.1
$CaPO_3F{\cdot}2H_2O$	4	0.0281	−6.0 ± 1.4	23.1 ± 9.60	41.2 ± 3.4

*Controls treated with distilled water for 144 hours at 37° C.; other sections treated with an unadjusted 2 per cent solution of fluorides for 144 hours at 37° C.; all sections were then exposed to acid solutions for 24 hours at 37° C.

†Mean and its standard error.

Critical ratios between weight losses of control and fluorine-treated dentin effected by succinic acid were *less* than 2 except for control versus NaF, control versus K_2PO_3F, NaF versus PbF_2, NaF versus K_2PO_3F, control versus PbF_2, CaF_2 versus K_2PO_3F, and PbF_2 versus $CaPO_3F{\cdot}2H_2O$.

Critical ratios between the accessibilities of the organic matrices of control and fluorine-treated dentin effected by succinic acid were *less* than 2 except for control versus PbF_2, CaF_2 versus PbF_2, CaF_2 versus K_2PO_3F, PbF_2 versus $CaPO_3F{\cdot}2H_2O$, and K_2PO_3F versus $CaPO_3F{\cdot}2H_2O$.

sections were treated as in the previous experiment except that they were decalcified in succinic acid. The solubility of the various dentinal sections in succinic acid was somewhat erratic, with the sections treated with calcium monofluorophosphate being more soluble than the control sections. The sections treated with lead fluorides were least soluble, with increasing solubility of the sections treated with sodium fluoride, potassium monofluorophosphate, calcium fluoride, control sections, and those treated with calcium monofluorophosphate. Statistical analysis of the mean weight losses of dentin exposed to succinic acid showed that the critical ratios between control and fluorine-treated dentin were less than 2 in 8 of 15 possible comparisons.

In contrast to the solubility of the inorganic portions of dentin where calcium monofluorophosphate-treated dentin was more soluble in succinic acid

than the control dentin, the accessibility of the organic dentinal matrix of the latter was greater than the organic matrix of dentin treated with calcium monofluorophosphate. The order of increasing accessibility of the groups of dentin exposed to succinic acid was as follows: calcium monofluorophosphate, control, calcium fluoride, potassium monofluorophosphate, sodium fluoride, and lead fluoride. Statistical analysis of the data for the accessibility of the organic matrices of the groups of dentin exposed to succinic acid showed that the critical ratios were less than 2 in 10 of 15 possible comparisons.

The actions of formic acid on the accessibility of the inorganic and organic components of control and treated dentin are summarized in Table VII. The dentin sections were treated as in the previous experiment except that they were decalcified in formic acid. In this case the control dentin was more soluble in the formic acid solution than were any of the other fluoride-treated sections. The groups of fluoride-treated dentin in order of increasing solubility in formic acid were as follows: sodium fluoride, lead fluoride, potassium monofluorophosphate, calcium monofluorophosphate, and calcium fluoride. Statistical analysis of the weight loss of the groups of control and treated dentin exposed to formic acid showed that the critical ratios between the different groups were greater than 2 in 10 of 15 possible comparisons.

TABLE VII

THE ACTION OF FORMIC ACID ON DENTIN TREATED WITH FLUORIDES

TYPE OF TREATMENT*	NUMBER OF SECTIONS	MEAN WEIGHT OF SECTIONS (GRAMS)	PER CENT WEIGHT CHANGE BY TREATMENT†	PER CENT WEIGHT LOSS†	ACCESSIBILITY OF THE ORGANIC DENTINAL MATRIX†
Control	4	0.0411	−1.7 ± 0.1	40.1 ± 5.6	15.8 ± 2.1
NaF	4	0.0474	−1.2 ± 0.2	14.6 ± 1.3	40.9 ± 9.2
CaF_2	4	0.0203	−2.2 ± 0.3	37.7 ± 3.3	32.1 ± 3.5
PbF_2	4	0.0348	+1.5 ± 0.7	15.4 ± 2.4	50.3 ± 4.1
K_2PO_3F	4	0.0415	−1.1 ± 0.3	17.4 ± 2.9	46.1 ± 7.1
$CaPO_3F{\cdot}2H_2O$	4	0.0378	−3.5 ± 0.6	21.8 ± 0.6	28.8 ± 0.7

*Controls treated with distilled water for 144 hours at 37° C.; other sections treated with an unadjusted 2 per cent solution of fluorides for 144 hours at 37° C.; all sections were then exposed to acid solutions for 24 hours at 37° C.

†Mean and its standard error.

Critical ratios between weight losses of the control and fluorine-treated dentin effected by formic acid were *greater* than 2 except for control versus CaF_2, NaF versus PbF_2, NaF versus K_2PO_3F, PbF_2 versus K_2PO_3F, and K_2PO_3F versus $CaPO_3F{\cdot}2H_2O$.

Critical ratio between the accessibilities of the organic matrices of control and fluorine-treated dentin effected by formic acid were *greater* than 2 except for NaF versus CaF_2, NaF versus PbF_2, NaF versus K_2PO_3F, CaF_2 versus $CaPO_3F{\cdot}2H_2O$, and PbF_2 versus $CaPO_3F{\cdot}2H_2O$.

The accessibility of the organic matrix of the control dentin decalcified with formic acid was less than any of the other groups of dentin exposed to the various fluorides. The increasing accessibility of the organic phase of the various groups of fluoride-treated dentin was as follows: calcium monofluorophosphate, calcium fluoride, sodium fluoride, potassium monofluorophosphate, and lead fluoride. Critical ratios between the weight losses of control and fluoride-treated dentin caused by formic acid were greater than 2 in 10 of 15 possible comparisons. The organic matrix of control dentin was least accessible, followed in increasing accessibility by the groups of dentin treated with calcium monofluorophosphate, calcium fluoride, sodium fluoride, potassium monofluoro-

phosphate, and lead fluoride. Statistical analysis of the accessibility of the organic matrices of the groups of dentin exposed to formic acid showed that the critical ratios were greater than 2 in 10 of 15 possible comparisons.

The effect of 2 inorganic acids on the inorganic and organic components of control and fluorine-treated dentin was also investigated. The results of the action of nitric acid upon control and fluoride-treated dentin are summarized in Table VIII. The dentin sections were treated as in the previous experiment except that they were decalcified in a nitric acid solution. The solubility of the various groups of dentin in the nitric acid solution varied considerably, with the sections of dentin treated with calcium fluoride being more soluble than the control sections treated only with water. As to the other groups of dentin, those treated with sodium fluoride were least soluble in the acid solution, with the solubility of the other groups of dentin increasing as follows: potassium monofluorophosphate, lead fluoride, and calcium monofluorophosphate. However, the critical ratios between weight losses of the various groups of dentin exposed to nitric acid were less than 2 in 10 of 15 possible comparisons.

TABLE VIII

THE ACTION OF NITRIC ACID ON DENTIN TREATED WITH FLUORIDES

TYPE OF TREATMENT*	NUMBER OF SECTIONS	MEAN WEIGHT OF SECTIONS (GRAMS)	PER CENT WEIGHT CHANGE BY TREATMENT†	PER CENT WEIGHT LOSS†	ACCESSIBILITY OF THE ORGANIC DENTINAL MATRIX†
Control	4	0.0268	−1.8 ± 0.5	17.3 ± 2.40	133.2 ± 38.8
NaF	4	0.0403	−0.8 ± 0.6	5.8 ± 0.76	56.7 ± 8.5
CaF_2	4	0.0240	−2.2 ± 0.4	19.4 ± 1.80	46.1 ± 3.8
PbF_2	4	0.0451	+1.6 ± 0.4	13.3 ± 4.80	41.5 ± 10.8
K_2PO_3F	4	0.0261	−1.2 ± 0.6	10.8 ± 1.60	72.6 ± 7.4
$CaPO_3F{\cdot}2H_2O$	4	0.0407	−4.4 ± 1.6	14.4 ± 3.30	44.8 ± 3.1

*Controls treated with distilled water for 144 hours at 37° C.; other sections treated with an unadjusted 2 per cent solution of fluorides for 144 hours at 37° C.; all sections were then exposed to acid solutions for 24 hours at 37° C.

†Mean and its standard error.

Critical ratios between weight losses of control and fluorine-treated dentin effected by nitric acid were *less* than 2 except for control versus NaF, control versus K_2PO_3F, NaF versus CaF_2, NaF versus $CaPO_3F{\cdot}2H_2O$, and CaF_2 versus K_2PO_3F.

Critical ratios between the accessibilities of the organic matrices of control and fluorine-treated dentin effected by nitric acid were *less* than 2 except for control versus CaF_2, control versus PbF_2, control versus $CaPO_3F{\cdot}2H_2O$, CaF_2 versus K_2PO_3F, PbF_2 versus K_2PO_3F, and K_2PO_3F versus $CaPO_3F{\cdot}2H_2O$.

The accessibility of the organic matrices of the control group of dentin exposed to nitric acid was approximately twice that of the most accessible group of dentin exposed to fluorides. The decreasing order of accessibility of the organic matrices of the groups of fluoride-treated dentin exposed to the nitric acid solution was as follows: potassium monofluorophosphate, sodium fluoride, calcium fluoride, calcium monofluorophosphate, and lead fluoride. Statistical analysis of the data for the accessibility of the matrices of the groups of dentin exposed to the nitric acid solution showed that the critical ratios between the various groups were less than 2 in 9 of 15 possible comparisons.

The effect of hydrochloric acid upon groups of control and fluoride-treated dentin is summarized in Table IX. The sections of dentin were treated as in the previous experiment except that they were decalcified in hydrochloric acid.

As to the solubility of the various groups of dentin, those treated with calcium fluoride and calcium monofluorophosphate were more soluble than even the control sections. All other groups of fluoride-treated dentin were less soluble than sections of the control group, with their order of decreasing solubility being: lead fluoride, sodium fluoride, and potassium monofluorophosphate. Statistical analysis of the percentage weight loss effected by hydrochloric acid showed that the critical ratios between the groups of dentin were greater than 2 in 8 of 15 possible comparisons.

TABLE IX

THE ACTION OF HYDROCHLORIC ACID ON DENTIN TREATED WITH FLUORIDES

TYPE OF TREATMENT*	NUMBER OF SECTIONS	MEAN WEIGHT OF SECTIONS (GRAMS)	PER CENT WEIGHT CHANGE BY TREATMENT†	PER CENT WEIGHT LOSS†	ACCESSIBILITY OF THE ORGANIC DENTINAL MATRIX†
Control	4	0.0437	−0.9 ± 0.4	11.8 ± 1.2	153.3 ± 32.0
NaF	4	0.0264	−0.9 ± 0.5	8.8 ± 1.9	66.5 ± 9.0
CaF_2	4	0.0328	−2.0 ± 0.2	16.7 ± 1.5	47.1 ± 1.9
PbF_2	4	0.0295	+1.5 ± 0.3	8.9 ± 1.6	67.4 ± 8.2
K_2PO_3F	4	0.0449	−1.6 ± 0.5	7.8 ± 0.8	57.9 ± 3.3
$CaPO_3F{\cdot}2H_2O$	4	0.0334	−3.6 ± 0.6	13.6 ± 1.1	51.1 ± 2.7

*Controls treated with distilled water for 144 hours at 37° C.; other sections treated with an unadjusted 2 per cent solution of fluorides for 144 hours at 37° C.; all sections were then exposed to acid solutions for 24 hours at 37° C.

†Mean and its standard error.

Critical ratios between weight losses of control and fluorine-treated dentin effected by hydrochloric acid were *greater* than 2 except for control versus NaF, control versus PbF_2, control versus $CaPO_3F{\cdot}2H_2O$, NaF versus PbF_2, NaF versus K_2PO_3F, CaF_2 versus $CaPO_3F{\cdot}2H_2O$, and PbF_2 versus K_2PO_3F.

Critical ratios between the accessibilities of the organic matrices of control and fluorine-treated dentin effected by hydrochloric acid were *greater* than 2 except for NaF versus PbF_2, NaF versus K_2PO_3F, NaF versus $CaPO_3F{\cdot}2H_2O$, CaF_2 versus $CaPO_3F{\cdot}2H_2O$, PbF_2 versus K_2PO_3F, PbF_2 versus $CaPO_3F{\cdot}2H_2O$, and K_2PO_3F versus $CaPO_3F{\cdot}2H_2O$.

The accessibility of the organic matrix of the control group of dentin exposed to hydrochloric acid was greater than the accessibility of any of the groups of dentin exposed to the various fluorides. In the various groups of fluoride-treated dentin exposed to hydrochloric acid the decreasing order of accessibility was: lead fluoride, sodium fluoride, potassium monofluorophosphate, calcium monofluorophosphate, and calcium fluoride. Statistical analysis of the data for the accessibility of the organic matrices of the treated and untreated groups of dentin exposed to hydrochloric acid showed that the critical ratios between the various groups were greater than 2 in 8 of 15 possible comparisons.

DISCUSSION

Most of the previous data concerned with the effect of fluorine, as well as other ions, on the acid solubility of enamel and dentin have been obtained by determining only the solubility of the inorganic components of powdered tissues in an acid solution containing no organic material. In this study, on the other hand, data have been obtained concerning the general effect of several fluorides upon both the accessibility of the inorganic and organic components of dentin in a variety of organic and inorganic acids that were buffered with a single inorganic buffer and in a solution containing protein and organic material, as in saliva. When the fluoride used for treatment of the sections from several teeth

was held constant but the sections from each tooth decalcified with a separate acid, it was found that solubility of the dentinal sections of the teeth within each treatment group did not differ significantly. A similar comparison of the accessibility of the organic dentinal matrices of these same teeth indicated that their critical ratios also did not differ significantly. When the dentin of different groups of teeth, divided according to their type of treatment, were compared, however, the solubility of their inorganic components differed significantly in the majority of possible comparisons. On the other hand, the accessibility of the organic matrices of dentin of the different groups of teeth, when compared in the same way, did not differ significantly in a majority of possible comparisons.

The high standard errors for both the solubility of the inorganic components of dentin and the accessibility of their organic matrices indicate that different acids, although at the same pH, act upon dentin in different ways. On the other hand, the standard errors calculated on the basis of the teeth rather than on the basis of the individual sections were much less, for the sections of each tooth had been treated in a similar manner.

The action of acids upon control and fluoride-treated dentin indicates that a complex relationship exists between the type of compound used to treat the dentin and the type of acid used to decalcify it. Sodium fluoride appears to cause a consistently greater decrease in the solubility of dentin than any other fluorides tested. In a few instances lead fluoride apparently decreased the solubility of the dentin more than did sodium fluoride. If the individual acids are considered, however, when acetic, butyric, and succinic acids were used, lead fluoride was apparently more effective in decreasing solubility than was sodium fluoride, but when lactic, citric, formic, hydrochloric, and nitric acids were used, sodium fluoride was apparently more effective than lead fluoride in decreasing the solubility of dentin. These differences in solubility are more apparent than real for critical ratios between weight loss of the different groups of dentin exposed to sodium and lead fluoride, indicating that no significant difference existed between the 2 fluorides when acetic, nitric, hydrochloric, and formic acid were used as the decalcifying acids. Thus, in most instances, the difference in the action of the 2 fluorides in depressing the solubility of dentin is more apparent than real.

The monofluorophosphates were also somewhat effective in decreasing the solubility of dentin, but calcium fluoride usually had the least apparent effect on the solubility of dentin, for the tissue treated with this compound did differ significantly from the controls only when acetic and butyric acids were utilized for decalcification.

When hydrochloric and nitric acid were utilized for decalcification, the accessibility of organic matrices of the control dentin was greater than the fluoride-treated dentin in a majority of the possible comparisons.

With regard to the action of the individual acid on control and fluoride-treated dentin, the organic matrices of the control sections were generally less accessible than the fluoride-treated sections when lactic, acetic, citric, succinic,

and formic acids were utilized for decalcification. When butyric and especially the inorganic acids were utilized for decalcification, the accessibility of the organic matrices was generally more than that of the fluoride-treated dentin. In comparing the effect of the various fluorides on the accessibility of the organic matrices of dentin, no consistent trend can be observed, for when one or another acid was used for decalcification, considerable variation was to be found in the accessibility of the organic matrices treated with any given fluoride. Also, when such data were subjected to statistical analysis, the significant differences between the accessibilities of the groups of dentin treated with the various fluorides varied considerably from one decalcifying acid to another.

These data have indicated that so far as the acid solubility of inorganic components and the accessibility of the organic dentinal matrix are concerned, a rather complex relationship exists between the dentinal tissue being treated, the type of fluoride used for treatment, and the acid used for decalcification.

CONCLUSIONS

The results show that generally the various fluorides affected the solubility of the inorganic components and the accessibility of the protein portions of dentin in significantly different ways when such dentin was exposed for a given period of time to several organic and inorganic acids in a buffered solution containing protein matter. However, these reactions appear to be more complex than had been supposed previously. Sodium fluoride was most consistent in depressing the solubility of the inorganic components of dentin, and in only a few instances lead fluoride was equally effective. Calcium fluoride depressed inorganic solubility least, but calcium and potassium monofluorophosphate depressed inorganic solubility significantly. The accessibility of the organic dentinal matrix was generally more in fluoride-treated dentin than in control dentin when exposed to organic acids but with the degree of accessibility reversed when it was exposed to inorganic acids.

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THE EFFECT OF PROTEIN COAGULANTS ON THE INORGANIC AND ORGANIC COMPONENTS OF HUMAN DENTIN

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INTRODUCTION

MANY chemical solutions have been applied to the calcified tissues of teeth in an attempt to diminish their tendency to decay.[1] Most of these compounds have been directed toward protecting the tissues from decalcification, but some of them may also have an effect upon the organic matrix. Silver nitrate, which is now known to react with both the inorganic and organic components of calcified tissues, was among the first compounds to be used for caries prophylaxis and therapy.[2] Miller,[4] in 1905, was among the first to study the effect of silver nitrate upon enamel and dentin in vitro, and he reported that it decreased the acid solubility of dentin, but not enamel (it has since been shown to affect also the solubility of enamel[3]), and therefore it could be used to arrest the progress of the disease, which it was thought to do by coagulating the content of and plugging the dentinal tubules. Miller also reported that oil of cinnamon, alcohol, zinc chloride, bichloride of mercury, and tannic acid did not protect dentin from decalcification. Some of these compounds do affect protein, and with the development of the theory that dental caries is primarily the result of the action of lytic bacteria upon the organic matrices of enamel and dentin,[5-7] such specific types of protein-coagulating compounds were applied to enamel with the hopes of decreasing its susceptibility to caries. Subsequently, it has been suggested that the enamel surface be impregnated with silver nitrate,[8, 9] zinc chloride,[8, 10] or potassium ferrocyanide,[10] singly or in combination,[11] to seal the ends of the organic pathways so as to exclude bacteria or their enzymes, and thereby prevent dental caries. Few attempts have been made to ascertain the specific action of such protein-coagulants upon both the inorganic and the organic components of the tooth tissues. A study has been conducted which indicates that zinc chloride and potassium ferrocyanide enhance the proteolysis of egg albumen by trypsin, but they had little or no effect on intact dentin treated in a similar way.[12] Since a method has been devised for determining the accessibility of tyrosine and histidine residues in dentinal protein which are indicative of accessibility of such protein for lysis by pepsin or carboxypeptidase,[13] it was felt that some information could be obtained as to the effect of such compounds upon human dentin. This report is concerned with the accessibility of both the organic and inorganic components of dentin after treatment with protein-coagulants and partial decalcification with several organic and inorganic acids.

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METHODS

Eight thin sections of dentin were cut with safe-side discs from each of 20 extracted and cleaned teeth. Pulp and cementum were removed from each section, after which they were dried to a constant weight in a vacuum desiccator at room temperature. The dried dentinal sections were then divided into 5 groups, each containing the sections of 4 teeth. One group was exposed to distilled water as control, and the others were exposed to water solutions of the protein-coagulants (2 per cent silver nitrate, 2 per cent neutral formalin, 40 per cent zinc chloride with 1 per cent polyoxyalkalene sorbitan monolaurate, and 20 per cent potassium ferrocyanide with 1 per cent polyoxyalkalene sorbitan monolaurate). The latter substance is a surface-reducing agent used by Palmer, Overstreet, and Sacks.[14] The dentin sections were exposed to the solutions for 10 days to elicit a maximum effect from their action. After exposure, the dentin sections were thoroughly washed in distilled water and dried to a constant weight in vacuo at room temperature. The control and treated dentin sections were then exposed to decalcifying solutions composed of 1 per cent peptone in phosphate buffer which had been adjusted to pH 3.0, respectively, with lactic, acetic, butyric, citric, succinic, formic, hydrochloric, or nitric acid. Each section of dentin from each tooth was decalcified in 10 ml. of each acid solution for the desired time. At the end of the decalcification period, the acids were removed rapidly, and their pH was checked. The sections were quickly neutralized in a saturated bicarbonate solution, thoroughly washed, and dried to a constant weight in vacuo at room temperature. After determining the weight loss of the dentinal sections, the accessibility of their tyrosine and histidine residues was determined according to the method of Burnett and Scherp.[13]

RESULTS

In the first experiment, the accessibility of the inorganic and organic components of dentin was determined after exposure to the protein-coagulants. Eight dentin sections from each of 20 teeth were exposed, respectively, to distilled water and to the solutions of silver nitrate, formaldehyde, zinc chloride, and potassium ferrocyanide for 240 hours at 37° C., in order to elicit a maximum effect. Each section from each tooth was decalcified separately in the 8 acid solutions, after which the accessibility of its organic matrix was determined by the method described. The results of this experiment are summarized in Table I. The treatment of the dentin sections with distilled water and potassium ferrocyanide resulted in a slight decrease in their weight. The sections treated with other solutions generally increased in weight, with those treated with silver nitrate increasing most. The means and their standard errors for percentage weight loss of the inorganic components and for the accessibility of organic matrices for each tooth were calculated on the basis of the individual sections, while those for the various groups of dentin, divided according to treatment, were calculated only on the basis of the individual teeth. Since each section from each tooth was exposed to a separate acid, such calcula-

tions were necessary to show the range of variation for sections from each tooth as well as the range of variation of the teeth. The percentage weight loss for the dentin sections within each group, divided according to the type of treatment, did not vary significantly within the group. This is indicated by the fact that the critical ratios (difference between the means divided by its standard

TABLE I

THE ACCESSIBILITY OF DENTIN TREATED WITH PROTEIN-COAGULANTS UPON EXPOSURE TO ORGANIC AND INORGANIC ACIDS

TYPE OF TREATMENT*	TOOTH NUMBER	NUMBER OF SECTIONS	MEAN WEIGHT OF SECTIONS IN GRAMS	PER CENT WEIGHT CHANGE BY TREATMENT†	PER CENT WEIGHT LOSS BY ACID†	ACCESSIBILITY OF THE ORGANIC DENTINAL MATRIX†
Control	49	8	0.0591	−1.1 ± 0.1	21.7 ± 5.2	36.3 ± 3.3
	50	8	0.0462	−0.3 ± 0.1	24.1 ± 5.6	36.1 ± 3.8
	51	8	0.0456	−1.0 ± 0.2	23.5 ± 5.1	37.9 ± 2.3
	52	8	0.0488	−1.0 ± 0.1	23.1 ± 5.9	34.5 ± 3.4
					23.1 ± 0.5	36.2 ± 0.7
$AgNO_3$ (2%)	57	8	0.0329	+10.3 ± 0.5	21.5 ± 5.6	Color interference
	58	8	0.0405	+9.0 ± 0.9	17.0 ± 3.5	Color interference
	59	8	0.0367	+9.3 ± 0.6	16.9 ± 3.6	Color interference
	60	8	0.0311	+8.3 ± 0.5	19.7 ± 4.0	Color interference
					18.7 ± 1.1	
HCHO (2%)	61	8	0.0637	+0.2 ± 0.1	22.3 ± 6.9	36.7 ± 3.0
	62	8	0.0481	0.0 ± 0.8	24.8 ± 5.1	40.1 ± 2.8
	63	8	0.0624	+0.2 ± 0.1	24.0 ± 5.8	35.0 ± 2.1
	64	8	0.0519	+0.2 ± 0.1	25.2 ± 4.7	37.4 ± 2.6
					24.0 ± 0.6	37.3 ± 1.0
$ZnCl_2$ (40%)	65	8	0.0478	+0.1 ± 0.1	26.7 ± 4.6	33.2 ± 3.0
	66	8	0.0495	0.0 ± 0.2	24.7 ± 4.4	35.2 ± 2.4
	67	8	0.0424	+0.1 ± 0.1	30.4 ± 5.2	30.4 ± 2.7
	68	8	0.0412	+0.1 ± 0.1	26.6 ± 4.9	33.3 ± 3.4
					27.1 ± 1.2	33.0 ± 0.9
$K_4Fe(CN)_6$ (20%)	69	8	0.0399	−1.5 ± 0.1	26.0 ± 7.3	42.9 ± 5.9
	70	8	0.0392	−1.2 ± 0.1	27.9 ± 6.7	42.5 ± 4.2
	71	8	0.0497	−1.4 ± 0.1	22.2 ± 5.3	44.1 ± 5.7
	72	8	0.0397	−0.9 ± 0.1	22.9 ± 4.1	40.5 ± 4.0
					24.7 ± 1.3	42.5 ± 0.7

*Period of exposure to water and protein-coagulants was 240 hours at 37° C.; one section from each tooth was exposed to lactic, acetic, butyric, citric, succinic, formic, hydrochloric, and nitric acid at pH 3.0 for 24 hours at 37° C.

†Mean and its standard error.

Critical ratios for teeth within a group: The critical ratios between percentage weight losses of the inorganic dentinal matrices and between accessibilities of the organic dentinal matrices were *less* than 2 for every possible comparison.

Critical ratios between different groups of teeth: (1) Critical ratios for percentage weight losses between different groups of teeth were *greater* than 2 except for control versus HCHO, control versus $K_4Fe(CN)_6$, $AgNO_3$ versus $K_4Fe(CN)_6$, HCHO versus $K_4Fe(CN)_6$, and $ZnCl_2$ versus $K_4Fe(CN)_6$. (2) Critical ratios for accessibilities of the organic matrices between different groups of teeth were *greater* than 2 except for control versus HCHO; $AgNO_3$ was not compared with the other compounds.

error) between weight losses of dentin from the teeth of each group did not exceed 2 for any of the possible comparisons. The mean percentage weight loss for the dentin of the various groups of teeth, divided according to treatment, varied significantly in one-half the possible comparisons.

The accessibility of the organic dentinal matrices did not differ significantly between the sections within any of the groups of teeth, for the critical ratios between the accessibility of the organic matrices of the sections within any

group were less than 2 for every possible comparison. However, the accessibility of the organic matrices of the dentin of the groups of teeth, divided according to treatment, did differ significantly in most instances, for the critical ratios were more than 2 for 5 of 6 possible comparisons.

In the remaining series of experiments, the action of individual acids upon the accessibility of the organic and inorganic components of dentin treated with protein-coagulants was determined. In these tests, each of the sections of dentin in a control or treated group was from a separate tooth. The control sections and treated sections were exposed, respectively, to water and protein-coagulants for 240 hours at 37° C.

The action of lactic acid upon treated and untreated dentin is summarized in Table II. Of the protein-coagulants, silver nitrate was the only one that decreased the solubility of the inorganic portion of dentin; the other protein-coagulants caused an apparent increase in the solubility of the dentin in lactic acid, but the critical ratios between weight losses of this dentin were less than 2 in 7 of 10 possible comparisons.

TABLE II

THE ACTION OF LACTIC ACID ON DENTIN TREATED WITH PROTEIN-COAGULANTS

TYPE OF TREATMENT*	NUMBER OF SECTIONS	MEAN WEIGHT OF SECTIONS IN GRAMS	PER CENT WEIGHT CHANGE BY TREATMENT†	PER CENT WEIGHT LOSS BY ACID†	ACCESSIBILITY OF THE ORGANIC DENTINAL MATRIX†
Control	4	0.0476	−1.0 ± 0.2	53.6 ± 2.9	30.1 ± 2.4
$AgNO_3$ (2%)	4	0.0392	+8.3 ± 0.6	41.0 ± 4.7	Color interference
HCHO (2%)	4	0.0430	0.0 ± 0.2	55.5 ± 4.5	25.7 ± 1.9
$ZnCl_2$ (40%)	4	0.0461	+0.2 ± 0.1	54.2 ± 2.4	24.3 ± 1.2
$K_4Fe(CN)_6$ (20%)	4	0.0429	−1.3 ± 0.2	55.0 ± 6.4	29.4 ± 1.6

*Controls treated with distilled water for 240 hours at 37° C.; other sections treated with a solution of the protein-coagulants for 240 hours at 37° C.; all sections were exposed to the acid solutions for 24 hours at 37° C.

†Mean and its standard error.

Critical ratios between weight losses of the various groups of treated and untreated dentin effected by lactic acid were *less* than 2 except for control versus $AgNO_3$, $AgNO_3$ versus HCHO, and $AgNO_3$ versus $ZnCl_2$.

Critical ratios between the accessibilities of the organic dentinal matrices of the various groups of control and treated dentin were *less* than 2 except for $ZnCl_2$ versus $K_4Fe(CN)_6$.

The accessibility of the organic dentinal matrices of dentin after treatment with silver nitrate could not be measured because of a color interference between silver nitrate and the diazoprotein. The accessibility of the organic matrices of the other groups of treated dentin was slightly less than that of the control group, but the critical ratios between the accessibilities of organic matrices of the different groups were less than 2 in 5 of 6 possible comparisons.

The action, under the same conditions, of acetic acid on dentin treated with protein-coagulants is summarized in Table III. With this acid, the solubility of control and treated dentin did not differ significantly, for in no case was the critical ratio between the various groups more than 2. Only in the case of formaldehyde-treated dentin did the accessibility of the organic dentinal matrices of any of the groups differ significantly, for the critical ratios between the accessibilities of the matrices were less than 2 in 3 of 6 possible comparisons.

TABLE III

THE ACTION OF ACETIC ACID ON DENTIN TREATED WITH PROTEIN-COAGULANTS

TYPE OF TREATMENT*	NUMBER OF SECTIONS	MEAN WEIGHT OF SECTIONS IN GRAMS	PER CENT WEIGHT CHANGE BY TREATMENT†	PER CENT WEIGHT LOSS BY ACID†	ACCESSIBILITY OF THE ORGANIC DENTINAL MATRIX†
Control	4	0.0540	−1.2 ± 0.3	28.6 ± 2.7	40.3 ± 2.4
$AgNO_3$ (2%)	4	0.0393	+8.5 ± 0.8	25.0 ± 1.3	Color interference
HCHO (2%)	4	0.0629	+0.3 ± 0.1	30.7 ± 4.8	30.9 ± 1.6
$ZnCl_2$ (40%)	4	0.0467	+0.05 ± 0.1	28.4 ± 3.7	41.3 ± 0.2
$K_4Fe(CN)_6$ (20%)	4	0.0439	−1.1 ± 0.2	28.8 ± 3.7	44.9 ± 2.3

*Controls treated with distilled water for 240 hours at 37° C.; other sections treated with a solution of the protein-coagulants for 240 hours at 37° C.; all sections were exposed to the acid solutions for 24 hours at 37° C.

†Mean and its standard error.

Critical ratios between weight losses of the various groups of control and treated dentin effected by acetic acid were *less* than 2 in each case.

Critical ratios between the accessibilities of the organic dentinal matrices of the various groups of control and treated dentin were *less* than 2 except for control versus HCHO, HCHO versus $ZnCl_2$, and HCHO versus $K_4Fe(CN)_6$.

The action, under the same conditions, of butyric acid on the accessibility of the organic and inorganic components of dentin treated with protein-coagulants is summarized in Table IV. The groups of dentin treated with formaldehyde, zinc chloride, and potassium ferrocyanide were apparently more soluble in butyric acid than was the control group, with only the silver nitrate–treated group being less soluble. Critical ratios between weight losses of control and the groups of treated dentin were more than 2 in 9 of 10 possible comparisons. The accessibility of the organic matrices of the various groups of dentin exposed to butyric acid varied only slightly, with the dentin exposed to formaldehyde being less accessible, and that exposed to potassium ferrocyanide being more accessible than the control dentin. Critical ratios between the accessibilities of the organic dentinal matrices of these groups of dentin were more than 2 in 4 of 6 possible comparisons.

TABLE IV

THE ACTION OF BUTYRIC ACID ON DENTIN TREATED WITH PROTEIN-COAGULANTS

TYPE OF TREATMENT*	NUMBER OF SECTIONS	MEAN WEIGHT OF SECTIONS IN GRAMS	PER CENT WEIGHT CHANGE BY TREATMENT†	PER CENT WEIGHT LOSS BY ACID†	ACCESSIBILITY OF THE ORGANIC DENTINAL MATRIX†
Control	4	0.0492	−0.7 ± 0.1	12.0 ± 0.8	50.0 ± 2.3
$AgNO_3$ (2%)	4	0.0417	+10.0 ± 0.9	9.9 ± 0.7	Color interference
HCHO (2%)	4	0.0568	+0.1 ± 0.2	18.5 ± 1.4	39.1 ± 0.4
$ZnCl_2$ (40%)	4	0.0411	+0.1 ± 0.2	19.9 ± 1.2	40.2 ± 4.1
$K_4Fe(CN)_6$ (20%)	4	0.0412	−1.2 ± 0.1	14.4 ± 0.7	51.6 ± 1.9

*Controls treated with distilled water for 240 hours at 37° C.; other sections treated with a solution of the protein-coagulants for 240 hours at 37° C.; all sections were exposed to the acid solutions for 24 hours at 37° C.

†Mean and its standard error.

Critical ratios between weight losses of the various groups of control and treated dentin effected by butyric acid were greater than 2 except for HCHO versus $ZnCl_2$.

Critical ratios between the accessibilities of the organic dentinal matrices of the various groups of treated and untreated dentin were *greater* than 2 except for control versus $K_4Fe(CN)_6$ and HCHO versus $ZnCl_2$.

The action, under the same conditions, of citric acid on the accessibility of the inorganic and organic matrices of treated and control dentin is summarized in Table V. In this case, control dentin treated with distilled water was more

soluble in citric acid than were any of the groups of dentin treated with protein-coagulants. Critical ratios between weight losses of the control and treated dentin were more than 2 in 7 of 10 possible comparisons. The accessibility of the organic matrices of the groups of dentin exposed to citric acid was variable, with some groups being more accessible and others less accessible than the control dentin. Critical ratios between the accessibilities of these groups of dentin were less than 2 in 4 of 6 possible comparisons.

TABLE V

THE ACTION OF CITRIC ACID ON DENTIN TREATED WITH PROTEIN-COAGULANTS

TYPE OF TREATMENT*	NUMBER OF SECTIONS	MEAN WEIGHT OF SECTIONS IN GRAMS	PER CENT WEIGHT CHANGE BY TREATMENT†	PER CENT WEIGHT LOSS BY ACID†	ACCESSIBILITY OF THE ORGANIC DENTINAL MATRIX†
Control	4	0.0606	−0.8 ± 0.1	11.2 ± 1.0	26.8 ± 1.8
$AgNO_3$ (2%)	4	0.0368	+10.0 ± 0.5	18.8 ± 2.5	Color interference
HCHO (2%)	4	0.0612	+0.2 ± 0.2	17.8 ± 1.8	39.9 ± 3.0
$ZnCl_2$ (40%)	4	0.0463	+0.2 ± 0.2	25.0 ± 1.3	25.9 ± 2.4
$K_4Fe(CN)_6$ (20%)	4	0.0423	−1.3 ± 0.1	21.5 ± 1.2	30.2 ± 8.4

*Controls treated with distilled water for 240 hours at 37° C.; other sections treated with a solution of the protein-coagulants for 240 hours at 37° C.; all sections were exposed to the acid solutions for 24 hours at 37° C.

†Mean and its standard error.

Critical ratios between weight losses of the various groups of control and treated dentin effected by the citric acid were *greater* than 2 except $AgNO_3$ versus HCHO, $AgNO_3$ versus $K_4Fe(CN)_6$, and $ZnCl_2$ versus $K_4Fe(CN)_6$.

Critical ratios between the accessibilities of the organic dentinal matrices of the various groups of treated and untreated dentin effected by citric acid were *less* than 2 except for control versus HCHO and HCHO versus $ZnCl_2$.

The action, under the same conditions, of succinic acid on dentin treated with protein-coagulants is summarized in Table VI. In this case, the inorganic matrices of the control dentinal sections were less soluble in the acid than were the sections treated with protein-coagulants, but the critical ratios between these groups were less than 2 in 7 of 10 possible comparisons. The accessibility of the organic dentinal matrices of the groups of dentin was variable, but not significantly so, for critical ratios between such accessibilities were not more than 2 for any possible comparison.

TABLE VI

THE ACTION OF SUCCINIC ACID ON DENTIN TREATED WITH PROTEIN-COAGULANTS

TYPE OF TREATMENT*	NUMBER OF SECTIONS	MEAN WEIGHT OF SECTIONS IN GRAMS	PER CENT WEIGHT CHANGE BY TREATMENT†	PER CENT WEIGHT LOSS BY ACID†	ACCESSIBILITY OF THE ORGANIC DENTINAL MATRIX†
Control	4	0.0414	−0.9 ± 0.2	17.2 ± 2.0	34.8 ± 3.9
$AgNO_3$ (2%)	4	0.0418	+9.3 ± 1.0	11.5 ± 1.0	Color interference
HCHO (2%)	4	0.0614	+0.2 ± 0.1	14.4 ± 1.4	38.7 ± 2.0
$ZnCl_2$ (40%)	4	0.0454	+0.2 ± 0.2	15.4 ± 1.5	33.4 ± 2.0
$K_4Fe(CN)_6$ (20%)	4	0.0424	−1.2 ± 0.3	16.2 ± 1.3	47.0 ± 8.2

*Controls treated with distilled water for 240 hours at 37° C.; other sections treated with a solution of the protein-coagulants for 240 hours at 37° C.; all sections were exposed to the acid solutions for 24 hours at 37° C.

†Mean and its standard error.

Critical ratios between weight losses of the various groups of control and treated dentin effected by succinic acid were *less* than 2 except for control versus $AgNO_3$, $AgNO_3$ versus $ZnCl_2$, and $AgNO_3$ versus $K_4Fe(CN)_6$.

Critical ratios between the accessibilities of the organic dentinal matrices of the various groups of control and treated dentin effected by succinic acid were *less* than 2 in all cases.

The effect of the last organic acid to be tested is summarized in Table VII. The dentin sections were treated as in the previous experiments except that they were decalcified in formic acid. In no instance did the solubility of the treated dentin significantly exceed that of the control group, for the critical ratios between the different groups of dentin did not exceed 2 in 8 of 10 possible comparisons.

TABLE VII

THE ACTION OF FORMIC ACID ON DENTIN TREATED WITH PROTEIN-COAGULANTS

TYPE OF TREATMENT*	NUMBER OF SECTIONS	MEAN WEIGHT OF SECTIONS IN GRAMS	PER CENT WEIGHT CHANGE BY TREATMENT†	PER CENT WEIGHT LOSS BY ACID†	ACCESSIBILITY OF THE ORGANIC DENTINAL MATRIX†
Control	4	0.0495	−1.1 ± 0.2	34.6 ± 3.2	36.3 ± 4.3
$AgNO_3$ (2%)	4	0.0344	+8.0 ± 0.7	26.5 ± 3.6	Color interference
HCHO (2%)	4	0.0533	+0.3 ± 0.2	34.1 ± 3.1	34.2 ± 1.6
$ZnCl_2$ (40%)	4	0.0445	−0.0 ± 0.2	35.8 ± 2.1	31.4 ± 2.6
$K_4Fe(CN)_6$ (20%)	4	0.0417	−1.4 ± 0.2	41.4 ± 4.5	33.5 ± 2.5

*Controls treated with distilled water for 240 hours at 37° C.; other sections treated with a solution of the protein-coagulants for 240 hours at 37° C.; all sections were exposed to the acid solutions for 24 hours at 37° C.

†Mean and its standard error.

Critical ratios between weight losses of the various groups of control and treated dentin effected by formic acid were *less* than 2 except for $AgNO_3$ versus $ZnCl_2$, and $AgNO_3$ versus $K_4Fe(CN)_6$.

Critical ratios between the accessibilities of the organic dentinal matrices of the various groups of control and treated dentin effected by formic acid were *less* than 2 in each case.

The accessibility of the organic dentinal matrices which had been exposed to formic acid differed little, for in no instance did the critical ratio between the various groups of dentin exceed 2.

The action of inorganic acids on treated and untreated dentin did not differ greatly from the action of organic acids except that, generally, the inorganic components of both control and treated dentin were less soluble in the inorganic acids than they were in the organic acids of the same pH. The data concerned with nitric acid are summarized in Table VIII. The dentin sections were treated as in the previous experiment except that they were

TABLE VIII

THE ACTION OF NITRIC ACID ON DENTIN TREATED WITH PROTEIN-COAGULANTS

TYPE OF TREATMENT*	NUMBER OF SECTIONS	MEAN WEIGHT OF SECTIONS IN GRAMS	PER CENT WEIGHT CHANGE BY TREATMENT†	PER CENT WEIGHT LOSS BY ACID†	ACCESSIBILITY OF THE ORGANIC DENTINAL MATRIX†
Control	4	0.0462	−0.8 ± 0.2	10.6 ± 0.8	36.5 ± 5.1
$AgNO_3$ (2%)	4	0.0340	+9.7 ± 1.3	10.2 ± 0.2	Color interference
HCHO (2%)	4	0.0556	+0.2 ± 0.1	10.4 ± 0.5	45.7 ± 1.4
$ZnCl_2$ (40%)	4	0.0492	+0.1 ± 0.1	15.8 ± 2.0	28.6 ± 3.3
$K_4Fe(CN)_6$ (20%)	4	0.0352	−1.3 ± 0.1	10.6 ± 0.9	56.2 ± 5.1

*Controls treated with distilled water for 240 hours at 37° C.; other sections treated with a solution of the protein-coagulants for 240 hours at 37° C.; all sections were exposed to the acid solutions for 24 hours at 37° C.

†Mean and its standard error.

Critical ratios between weight losses of the various groups of control and treated dentin effected by nitric acid were *less* than 2 except for control versus $ZnCl_2$, $AgNO_3$ versus $ZnCl_2$, HCHO versus $ZnCl_2$, and $ZnCl_2$ versus $K_4Fe(CN)_6$.

Critical ratios between the accessibilities of the organic dentinal matrices of the various groups of control and treated dentin effected by nitric acid were *greater* than 2 except for control versus HCHO and control versus $ZnCl_2$.

decalcified in nitric acid. Critical ratios between weight losses of the control and treated groups exposed to nitric acid were less than 2 in 6 of 10 possible comparisons. The accessibility of the organic dentinal matrices of dentin treated with formaldehyde and potassium ferrocyanide exceeded that of control dentin.

Critical ratios between the accessibilities of the organic dentinal matrices of these groups of dentin were more than 2 in 4 of 6 possible comparisons.

The results of the action, under the same conditions, of hydrochloric acid are summarized in Table IX. In this case, the results were somewhat comparable to those obtained when nitric acid was used. Only when silver nitrate was used to treat dentin were the inorganic components of any of the groups of dentin less soluble than those of the control group. On the other hand, dentin treated

TABLE IX

THE ACTION OF HYDROCHLORIC ACID ON DENTIN TREATED WITH PROTEIN-COAGULANTS

TYPE OF TREATMENT*	NUMBER OF SECTIONS	MEAN WEIGHT OF SECTIONS IN GRAMS	PER CENT WEIGHT CHANGE BY TREATMENT†	PER CENT WEIGHT LOSS BY ACID†	ACCESSIBILITY OF THE ORGANIC DENTINAL MATRIX†
Control	4	0.0508	-1.2 ± 0.2	9.9 ± 1.3	34.9 ± 2.9
$AgNO_3$ (2%)	4	0.0413	$+10.0 \pm 1.5$	7.8 ± 1.0	Color interference
HCHO (2%)	4	0.0600	$+0.2 \pm 0.1$	11.3 ± 2.3	44.4 ± 1.4
$ZnCl_2$ (40%)	4	0.0435	-0.5 ± 0.2	17.9 ± 1.8	39.1 ± 4.4
$K_4Fe(CN)_6$ (20%)	4	0.0388	-1.2 ± 0.2	10.4 ± 1.2	47.4 ± 6.7

*Controls treated with distilled water for 240 hours at 37° C.; other sections treated with a solution of the protein-coagulants for 240 hours at 37° C.; all sections were exposed to the acid solutions for 24 hours at 37° C.

†Mean and its standard error.

Critical ratios between weight losses of the various groups of control and treated dentin effected by hydrochloric acid were *less* than 2 except for control versus $ZnCl_2$, $AgNO_3$ versus $ZnCl_2$, HCHO versus $ZnCl_2$, and $ZnCl_2$ versus $K_4Fe(CN)_6$.

Critical ratios between the accessibilities of the organic dentinal matrices of the various groups of control and treated dentin were *less* than 2 except for control versus HCHO.

with zinc chloride was almost twice as soluble as control dentin. Critical ratios between weight losses of these groups of dentin were less than 2 in 6 of 10 possible comparisons. The accessibility of the organic matrices of the groups of dentin varied considerably, with the dentin treated with formaldehyde and potassium ferrocyanide being more accessible, and that treated with zinc chloride being less accessible than control dentin. Critical ratios between the accessibilities of the organic matrices of this dentin were less than 2 in 5 of 6 possible comparisons.

DISCUSSION

While protein-coagulants have been applied topically to tooth tissues from time to time in the hopes of decreasing their susceptibility to dental caries, few studies have been made of the specific effect of such compounds upon both the organic and inorganic components of the tooth tissues. Gottlieb[8] indicated that silver nitrate, potassium ferrocyanide, or zinc chloride reacted with the organic matrix of the calcified tooth tissues, but they did not indicate what effect such a reaction had upon the accessibility of the treated tissue to proteolytic enzymes. The study of Dannenberg and Bibby[12] was among the first that considered whether treatment of tooth tissues with a protein-coagulant increased or

decreased their accessibility to proteolytic enzymes, but their method of measuring changes in the organic matrix of dentin gravimetrically was not a particularly sensitive one. This method could only measure whether complete dissolution of the organic matrix occurred and did not indicate such possible lesser changes that seem to occur in dentin before its complete lysis. The investigations of Burnett and Scherp[13] have indicated that decalcification with organic or inorganic acids liberates the organic matrix of dentin in proportion to the degree of decalcification, and that it is essentially not accessible before liberation during decalcification. This investigation has been directed toward ascertaining the effects of protein-coagulants on the solubility of the inorganic portions of dentin, together with their effect on the accessibility of the organic matrix of such dentin when it is liberated with organic or inorganic acids. Minor variations occurred in the effect of these substances on this tissue, but in no instance, even after long exposure, did they have an overwhelming, or even decisive, effect upon the accessibility of either the organic or inorganic components of such tissue when they were decalcified with organic and inorganic acids. There is, however, some evidence that the solubility of treated and untreated dentin varies with the type of acid used for decalcification, even though the acids are at the same pH. These findings emphasize the fact that due consideration must be given to the type of compound used to treat dentin as well as to the type of acid with which it is decalcified. The different acids also sometimes affected the accessibility of the organic matrix of treated and untreated dentin in different ways, but in no instance did the protein-coagulants seriously interfere with or depress the accessibility of the organic matrix when it was liberated during decalcification. In some instances, there were more accessible tyrosine and histidine groups in dentin after treatment with protein-coagulants and decalcifying acid than there were when it was exposed to distilled water before partially decalcifying it. Such findings might indicate that the treatment of tooth tissue with various compounds which coagulated it or were strongly reducing, such as potassium ferrocyanide, might actually make it more accessible to lytic action in the dental carious process. Some of the keratins, for instance, require exposure to reducing substances before they can be lysed by proteolytic enzymes. An example of such a situation is the digestion of the keratin of wool fibers by an enzyme in the intestine of the clothes moth in the presence of H_2S liberated by intestinal bacteria.[15]

CONCLUSIONS

The protein-coagulants, silver nitrate, zinc chloride, and potassium ferrocyanide, had no overwhelming effect upon either the acid solubility of the inorganic portions or the accessibility of organic matrix of dentin even after its decalcification. While the protein-coagulants did not interfere with the accessibility of the organic dentinal matrix, they did sometimes effect the liberation of more accessible tyrosine and histidine residues than were liberated from control dentin. The solubility of the inorganic component and the accessibility of the organic matrix of dentin sometimes varied with the type of acid used for decalcification.

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EFFECTS OF ASHED FOODSTUFFS ON DENTAL DECAY IN HAMSTERS

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THIS investigation into the effect of inorganic constituents of a natural diet on tooth integrity was prompted by previous regional differences noted in dental caries prevalence in human beings[1] and animal[2, 3] populations. In in vitro and in vivo experiments, the cariogenic and cariostatic influences of individual elements have been studied, but little attention has been paid to the effects of mixtures of elements in the proportion in which they occur in natural foodstuffs.

Manly and Bibby[4] collaborated in the publication of independent investigations in which they tested 147 compounds on their effectiveness in reducing the acid solubility of tooth enamel. Twenty-seven of these substances were found to be more effective than 0.1 per cent NaF. In another in vitro experiment, it was noted by Stephan[5] that the growth of oral lactobacilli was stimulated by the addition of magnesium, manganese, and potassium to the culture medium, and was inhibited by the addition of such elements as copper, cobalt, nickel, molybdenum, and fluorine. On the basis of Stephan's work, Dreizen, Spies, and Spies[6] investigated the cariostatic properties of copper and cobalt when added to samples of human saliva. They added 20.0 μg of copper and 19.7 μg of cobalt to 10 c.c. aliquots of stimulated saliva obtained from patients with rampant caries. Acid production was inhibited to some degree in all samples. Forbes and Smith[7] observed that copper at a concentration of 3 to 4 mg. per 100 ml. of stimulated saliva, containing sucrose, could completely inhibit acid production. Nickel, silver, gold, mercury, platinum, palladium, and zinc also inhibited the lowering of saliva pH, but cobalt, manganese, aluminum, and iron had little or no effect.

In in vivo experiments, fluorine,[8] vanadium,[9, 10] and strontium have been shown to exert a caries-inhibiting effect in hamsters and rats. On the other hand, magnesium, silver, and copper were reported to have no effect on caries development, but cadmium,[12] zinc,[10] barium,[10] and thallium were observed to be cariogenic.

In the studies mentioned previously, attempts were made to single out an element or compound which might influence the development of dental caries. It should be pointed out, however, that these elements ordinarily are obtained as mixtures of organic and inorganic compounds in the water, food, and condiments of the diet. It is important to know, therefore, whether the natural presence and ratios of all the mineral elements in foods and dietaries affect the development of dental caries. An approach to this problem can be made by studying the effects of the ash of foodstuffs on the development of caries.

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MATERIALS AND METHODS

One hundred pounds of Diet A (Table I) was charred over a direct flame, and then ashed at 510° C. in a muffle furnace. This ash was then used in the preparation of Diet B (Table I). The total ash contents of Diets A and B were 3.46 per cent and 6.42 per cent, respectively.

TABLE I

COMPOSITION OF EXPERIMENTAL DIETS

	DIET A	DIET B
Ground whole corn	63	61
Whole milk powder	30	29
Alfalfa meal	6	6
Sodium chloride	1	1
Ash prepared from Diet A	0	3
Total	100	100

From the time of the birth of their young, the mothers of 24 hamsters were offered Diet A, and the mothers of 17 hamsters were given Diet B ad libitum. The young were weaned at 21 days, placed in individual wooden cages, and continued on the same diets until 20 weeks old. Distilled water was given ad libitum. No gross evidence of abnormalities was observed in either of the groups. At the end of the test period, the hamsters were sacrificed, and the jaws removed and stripped of soft tissue. Caries were scored and charted by the Johansen method,[13] using a binocular dissecting microscope.

RESULTS

Because of the nature of the diet used, there was a low incidence of caries in both groups. However, the incidence and extent of the caries were significantly less in the group which received the ash-supplemented diet. The pits and fissures of the teeth of the supplemented group were relatively smooth and lustrous. Of the 17 hamsters which had received the ash-supplemented diet, 13 had no lesions and 4 had one small lesion each (average 0.01). Of the 24 hamsters which had received the unsupplemented diet, 4 had no lesions, and the remaining 20 had scores ranging between 0.05 and 0.95 (average 0.18).

It is apparent that the ash of these foodstuffs exerted a caries-inhibitory effect when mixed in the diet and fed to hamsters from birth. This confirms the results of a similar experiment performed in these laboratories.[14, 15] Other investigators have added natural ash mixtures to synthetic diets rather than to natural diets and report similar cariostatic effects.[16, 17] It remains to be shown whether the caries inhibiting effect of the food ash is due to a local action in the oral cavity or is a systemic (nutritional) effect.

SUMMARY

1. The ash of a diet containing whole corn, whole milk powder, and alfalfa meal was mixed with an equivalent amount of the same diet, and fed to hamsters for 20 weeks, beginning at birth. A second group was fed this diet without ash supplementation.

2. The caries score of the group fed the ash-supplemented diet was 0.01. The score of the hamsters fed the control diet was 0.18. Thus, the ash of these natural foodstuffs exerted a caries-inhibitory action when fed in the diets of hamsters.

3. It is not known whether this action against tooth decay is exerted locally or systemically.

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AN ANALYSIS OF THE BUFFER SYSTEMS IN SALIVA

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THE significance of any analysis of the buffer systems of saliva depends largely on the definition of the term "saliva" and on the methods used in the determination of buffer capacity. The secretion obtained from any one of the salivary glands differs considerably from that of any other and from that obtained by allowing the mixed secretions to collect in the mouth (the so-called resting saliva). Furthermore, activated saliva, obtained by chewing paraffin wax or other suitable material, is different again. The carbon dioxide pressure in the gas phase in contact with the secretions is of utmost importance to the salivary pH, a fact stressed by Sellman.[8] In a solution of constant bicarbonate concentration the pH will vary according to the partial pressure of carbon dioxide in the gas phase in contact with the solution. Hence, on removal of saliva from the oral environment, its pH will alter if the partial pressure of carbon dioxide is not maintained at that in the mouth (neglecting metabolic effects of microorganisms in the saliva). Under conditions of carbon dioxide pressure which are likely to exist near the orifices of the salivary glands, it is mainly the amount of bicarbonate secreted which will determine the pH of the saliva as it enters the oral cavity from any given duct.

Several investigators have attempted to relate the buffer capacity of saliva to caries susceptibility, viz., Dreizen, Mann, Cline, and Spies,[4] Rae and Clegg,[7] Sellman,[8] and Dewar.[3] The buffering power of dentobacterial plaques also has been given some attention by Stralfors[10] and by Fosdick,[5] who believe that plaque has an important buffering action.

The aim of the work reported here was to determine the relative importance of various buffer systems in human saliva, namely, bicarbonate, phosphate, protein, mucin, and bacteria, in the hope that this information would give greater meaning to buffer capacity tests and more background knowledge of the mechanism of acid neutralization in the mouth.

METHODS

Collection of Saliva for Buffer Tests.—Activated saliva: Unless stated otherwise, the subject chewed a piece of paraffin wax for 5 minutes and the activated saliva was collected under paraffin oil in a 6 inch by 1 inch test tube. Resting saliva: The saliva which collects in the floor of the mouth when at rest was designated "resting" saliva. It was collected in tubes kept in ice.

Titration of Salivary Samples.—To 2.0 ml. distilled water, under a seal of paraffin oil, was added 1.0 ml. saliva with the delivery end of the pipette beneath the paraffin seal. During the titration with standardized acid, the tip of the burette was placed under the paraffin seal and the sample was frequently

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stirred with a glass rod. A glass electrode assembly was used (Model B Potentiometer, Jones, Melbourne, Australia) to determine the pH after each addition of definite quantities of acid.

Carbon Dioxide-Free Air.—For the removal of the bicarbonate-carbonate system, carbon dioxide-free air was used by passing compressed air through a trap containing 20 per cent (w/v) KOH, thence through the diluted saliva or the solution under examination via a Pasteur pipette, which contained a cotton wool plug to prevent carry-over of any droplets of the alkali.

Preparation of Salivary Mucoid.—The method adopted was the one described by Knox and Still[6] in which the mucoid was precipitated by citric acid.

Estimation of Mucoid.—The ACRA titer (acid, Congo red, alcohol) method of Burnet[1] was used.

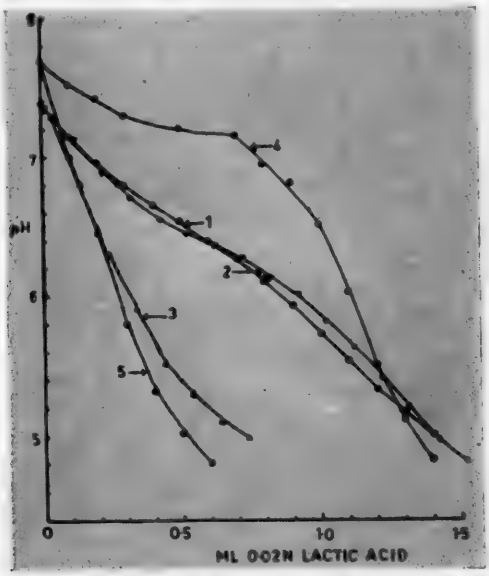


Fig. 1.—Effect on the buffer curve of removal of bicarbonate from saliva. System: saliva 1.0 ml., H_2O 2.0 ml., in each case. Curve 1: titration of saliva under paraffin oil; Curve 2: saliva treated as in 1, pH adjusted to original with NaOH, then titrated again with acid; Curve 3: treatment as for 2, bicarbonate removed at pH 5.0, pH readjusted with NaOH and titrated again with acid; Curve 4: saliva titrated, no paraffin oil seal, with carbon dioxide-free air bubbled through the solution; Curve 5: as for 4, after pH was readjusted; no passage of air during second titration.

EXPERIMENTAL

Removal of the Bicarbonate System From Saliva.—When a sample of activated saliva is titrated with lactic acid under a seal of paraffin oil to a pH value below 5.0, addition of NaOH restores the pH to its original value, and a subsequent titration with acid gives a curve very similar to the first one. If carbon dioxide-free air is bubbled through the solution for 5 minutes when the pH is below 5.0, the bicarbonate system is removed. Adjustment of the pH then, with NaOH as before, followed by titration with acid, gives a curve which represents the buffer systems of saliva without bicarbonate. Examples of these curves are given in Fig. 1 (Curves 1, 2, and 3).

When carbon dioxide-free air is passed through the saliva during the titration, carbon dioxide is removed continually and a curve (Fig. 1, Curve 4) is obtained, which is quite different in shape from that of Curve 1, although a similar quantity of acid was required in both cases to lower the pH to 5.0. Discontinuation of the passage of air, readjustment of pH of the saliva with NaOH, and then titration with acid gives a curve for the buffer systems without bicarbonate (Fig. 1, Curve 5). This curve should be similar to Curve 3, and the difference between these curves is due in this case to the different quantities of lactic acid which had been added to the samples prior to these titrations.

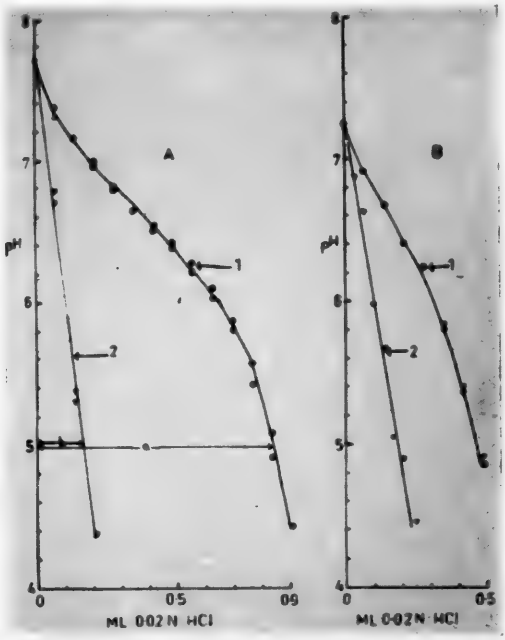


Fig. 2.—Effect of aeration time on bicarbonate removal from saliva. *A* and *B*: samples of saliva of different bicarbonate content; Curve 1: titration of saliva under paraffin oil seal; Curve 2: titration after aeration at pH below 5.0 for 5 min. ▽——▽ and 25 min. ▼——▼. Value (a – b) represents the bicarbonate system in the saliva.

Tests were made to check the efficiency of carbon dioxide-free air in removing the bicarbonate system. Samples of saliva were titrated for total buffer capacity and then carbon dioxide-free air was bubbled through the solution at pH below 5.0 for 5 minutes or 25 minutes. Fig. 2 shows that no significant difference occurred if the time of air passage was increased from 5 to 25 minutes.

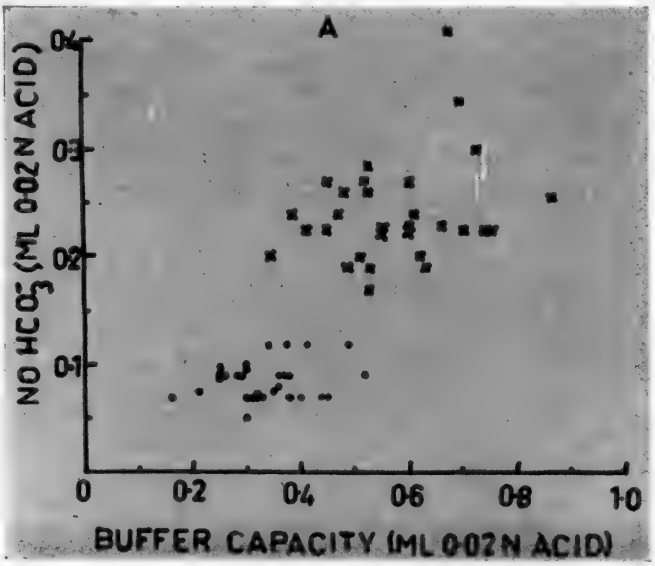
Buffer Curves for Phosphate and Bicarbonate.—Under the conditions of pH usually existing in saliva, the phosphate present will be largely in the form of $H_2PO_4^-$ and $HPO_4^=$ with small amounts of $PO_4^{\equiv}$. It was deemed advisable to study mixtures of phosphate buffer and bicarbonate in the concentrations known to occur in saliva to determine whether the technique used for bicarbonate removal from saliva held for this known system. With 3 concentrations of

bicarbonate and the same concentration of phosphate, the titration curves shown in Fig. 3 were obtained. These indicate that removal of bicarbonate by this method was independent of its concentration. When these mixtures were titrated without a paraffin seal and carbon dioxide-free air was used during the titration, the curves were displaced in the same manner shown for saliva in Fig. 1. After readjustment of pH with NaOH and further titration with acid (no air used), the same curve for the phosphate buffer was obtained in each case. Other tests in which the phosphate buffer concentration was altered, but the bicarbonate concentration was kept constant, again showed that, after carbon dioxide removal at a low pH value, only phosphate remained, and it did not influence bicarbonate removal.

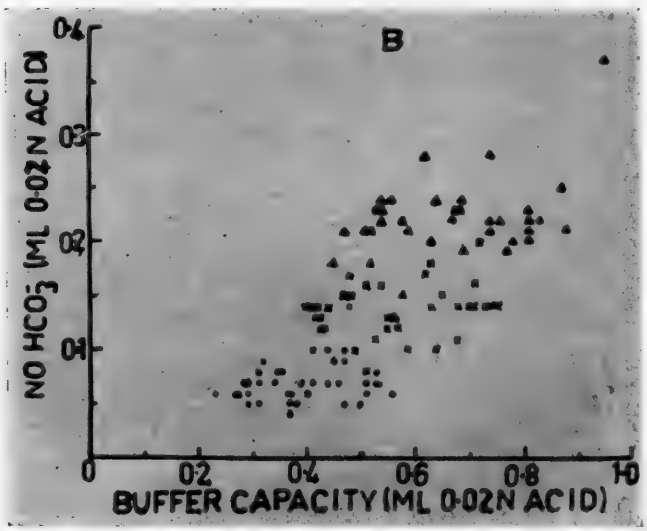
Fig. 3.—Titration of mixtures of bicarbonate and phosphate. System: bicarbonate-phosphate mixture 1.0 ml., H_2O 2.0 ml., titrated under paraffin oil ——— before and - - - - after removal of bicarbonate by carbon dioxide-free air. Phosphate in each case was 0.01 M Na_2HPO_4–KH_2PO_4 buffer, pH 7.2; bicarbonate concentrations x——x 0.01 M; ⊙——⊙ 0.013 M and ●——● 0.02 M; ▼——▼ phosphate buffer 0.01 M alone.

The Importance of the Bicarbonate System in Activated Saliva.—With the aid of the method just described for removing the bicarbonate-carbonate system from saliva, it was possible to obtain some idea of the importance of this system as a salivary buffer. The buffer capacity was determined by titration with lactic acid under a seal of paraffin oil. Then, on removal of the bicarbonate system, the other buffers were titrated with acid after readjustment of the pH with

NaOH. In Fig. 4, *A*, the buffer capacities for saliva of 30 subjects are plotted against the buffer capacities for the same samples in the absence of bicarbonate. Although the total buffer capacity for the range pH 7.0 to 6.0 varied threefold,



A.



B.

Fig. 4.—Comparison of buffer capacity before and after bicarbonate removal. *A*: titrations with lactic acid and NaOH to readjust pH; 30 subjects in group; values for pH 7.0 to 6.0, ●; pH 7.0 to 5.0, x. *B*: titrations with HCl using $Ba(OH)_2$ to readjust pH; 36 subjects in group; values for pH 7.0 to 6.0, ●; pH 7.0 to 5.0, x; pH 7.0 to 4.0, ▲.

the buffer capacities were less variable in the absence of bicarbonate (this was not so marked for the pH range 7.0 to 5.0). In other words, it is mainly the variation of bicarbonate which is responsible for the different buffer capacity values obtained with saliva from different subjects, for the phosphate buffer system and other systems, if any, appear to remain reasonably constant. The variation in "phosphate buffer" was more marked for the range pH 7.0 to 5.0 due to the fact that lactic acid is too weak an acid to titrate the phosphate system. This is indicated clearly in Fig. 5, where the curves are shown for saliva titrated with HCl and lactic acid. Similar curves were obtained for total buffering power but, after removal of bicarbonate, lactic acid did not give a linear relationship as did HCl when the pH approached 5.0.

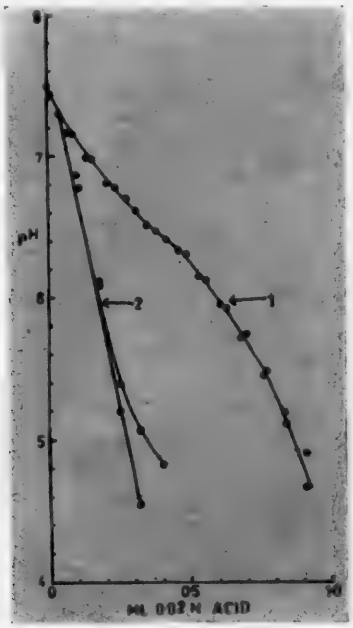


Fig. 5.—Saliva titrated with hydrochloric and lactic acids. Legend: ●——● lactic acid; ⊙——⊙ hydrochloric acid; Curve 1: before bicarbonate removal; Curve 2: after bicarbonate removal.

Another group of 36 subjects was studied in the same manner using HCl and $Ba(OH)_2$ instead of lactic acid and NaOH, respectively. Baryta was found to give the same results as NaOH when it was used to readjust the pH of a sample of saliva, and its use avoided the risk of addition of bicarbonate or carbonate to the sample. The results obtained are shown in Fig. 4, *B*, and these again emphasize the role of bicarbonate as a salivary buffer. Other information was obtained from these titrations with HCl. The difference between the volume of HCl required to change the pH from its original value to 5.0 before and after removal of the bicarbonate-carbonate system is a measure of the bicarbonate system in the saliva (Fig. 2, *A*, *B*). Another method of estimation of the bicar-

bonate-carbonate system is by gasometric analysis, using the Van Slyke apparatus. After suitable correction for temperature and pressure and also for dissolved carbon dioxide and air, the volume of carbon dioxide can readily be expressed in terms of milliliters of standard acid. This was done for the saliva of the 36 subjects and a correlation was made of these 2 methods. The titrations and the gasometric analysis of a sample of saliva were completed within 20 minutes of its collection. Fig. 6 contains the results for these 2 methods expressed in the same units (Ml. 0.02 N HCl). It can be seen that the Van Slyke method constantly gave higher values than the titration method and, further, that at the top end of the range the difference was accentuated. No adequate explanation for this can be offered.

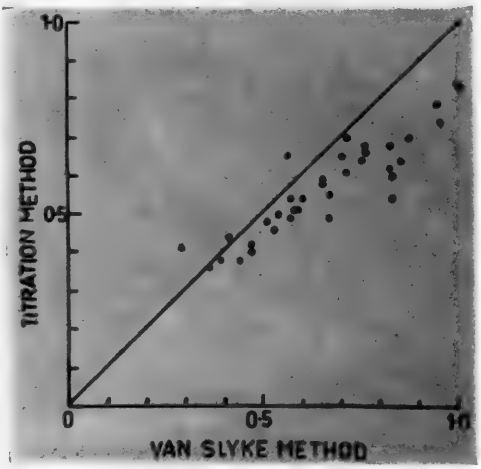


Fig. 6.—Comparison of Van Slyke and titration methods for bicarbonate estimations. Results expressed in ml. 0.02 N HCl.

The Effect of Dialysis on Buffer Capacity.—On dialysis of activated saliva, phosphate and bicarbonate are removed together with other electrolytes; the larger molecules, such as proteins and mucoids, remain inside the Cellophane sac with the bacteria. When saliva in such a sac was dialyzed against distilled water for 4 hours at 4° C., all the phosphate and 90 per cent of the bicarbonate were removed as determined by colorimetric and gasometric analysis, respectively. The contents of the sac were titrated before and after this residual bicarbonate was removed and the buffering capacity was determined. The results are presented in Fig. 7 and it is obvious that dialysis greatly reduced the buffer capacity. When the small amount of bicarbonate remaining in the sample was removed, the curve obtained was the same as that for distilled water. These observations thus reveal the absence of any significant degree of buffering power in the bacteria, mucoids, and proteins in the sample of activated saliva.

Buffer Capacity of Salivary Mucoid.—Salivary mucoid was precipitated from saliva by citric acid (Knox and Still[6]). After washing with 0.85 per cent NaCl the mucoid was redissolved in 0.5 per cent Na_2CO_3 and the buffer curve

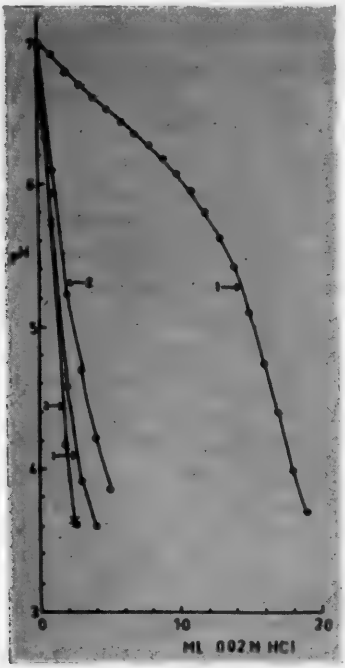


Fig. 7.—The effect of dialysis on the buffer systems in saliva. Legend: ⊙——⊙ saliva kept 4 hr. at 4° C., undialyzed; ●——● saliva after dialysis for 4 hr. at 4° C. against distilled water; x——x distilled water; Curve 1: before bicarbonate removal; Curve 2: after bicarbonate removal.

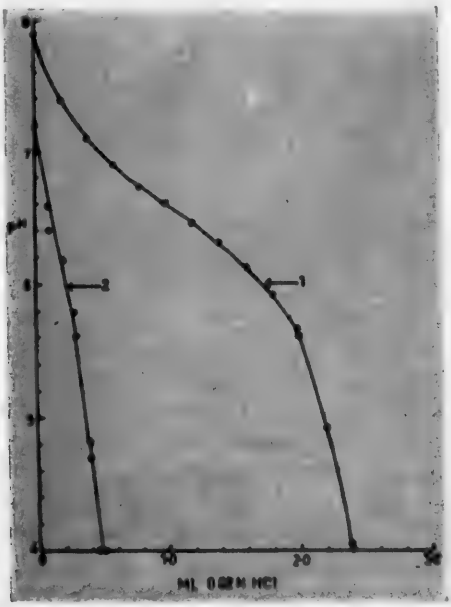


Fig. 8.—Titrations of salivary mucoid. Legend: ⊙——⊙ mucoid in 0.5 per cent Na_2CO_3, 1.0 ml.; H_2O, 2.0 ml.; ●——● 0.5 per cent Na_2CO_3, 1.0 ml.; H_2O, 2.0 ml. Curve 1: before bicarbonate removal; Curve 2: after bicarbonate removal; mucoid solution had ACRA titer of 2.

determined before and after removal of the carbonate, as described previously. A solution of 0.5 per cent Na_2CO_3 was treated in like manner. The results (Fig. 8) show that salivary mucoid has no buffering action between pH 7.0 and 4.0. Similar results were obtained with mucoid solutions of different concentrations (ACRA titers up to 4).

When a solution of mucoid was added to a mixture of phosphate buffer and bicarbonate, the buffering power increased. However, when the bicarbonate-carbonate system was removed, the buffer curve obtained over the range pH 7.0 to 4.0 was the same as that for the phosphate buffer alone. This again indicated the lack of buffering action of the mucoid, the increase in buffering power before removal of bicarbonate being due to the carbonate used to redissolve the mucoid.

Yet another method was used to test the ability of mucoid to neutralize acid. If salivary mucoid is a buffer, its addition to HCl should alter the pH of the solution over a period of days. When this test was carried out with a large amount of mucoid and 3.0 ml. HCl, no significant change in pH occurred during 7 days at 4° C. (Table I).

TABLE I

EFFECT OF THE ADDITION OF SALIVARY MUCOID ON THE pH OF HYDROCHLORIC ACID SOLUTIONS

TIME AT 4° C.	pH							
	EXPERIMENT 1		EXPERIMENT 2		EXPERIMENT 3		EXPERIMENT 4	
	HCl	HCl + MUCOID	HCl	HCl + MUCOID	HCl	HCl + MUCOID	HCl	HCl + MUCOID
0	1.93	1.89	2.91	2.90	3.92	3.89	4.25	4.23
4 hr.	–	1.90	2.83	2.96	3.95	3.90	–	–
1 day	–	–	–	–	–	–	4.2	4.22
4 days	–	1.91	2.82	2.94	3.93	4.1	4.17	4.21
7 days	–	–	–	–	–	–	4.22	4.25

Mucoid was precipitated from saliva by citric acid, washed twice with NaCl, and excess water was removed by drying between filter paper.

Quantity of mucoid added to the HCl solutions: Experiments 1, 2, and 3—approximately 25 mg. mucoid per milliliter HCl; Experiment 4—approximately 200 mg. mucoid per milliliter HCl.

Salivary Sediment and Plaque.—On centrifugation of activated saliva a sediment is obtained which consists largely of bacteria. Stralfors[10] tested this material for buffering power and found it to be similar to plaque material. In view of the possibility of adsorption by the cells of inorganic buffers from saliva, tests were made to determine what proportion of the buffering power of the sediment was due to such a process. Titration of salivary sediment before and after removal of the bicarbonate system gave the results shown in Fig. 9. Bicarbonate, therefore, plays a variable role in the buffering power of this material. By dialyzing the material at 4° C. against distilled water, both phosphate and bicarbonate, if present, would be removed. If phosphate were adsorbed by the cells, then dialyzed sediment would give a titration curve steeper than that given by undialyzed sediment after removal of bicarbonate. That this was not obtained with most of the samples tested suggests that phosphate does not contribute to the buffering power of this system.

Dentobacterial plaque, obtained in a manner similar to the one described by Stralfors, did not show the presence of bicarbonate. The buffering system seems to be due to the organisms contained within the plaque.

The Effect of Incubation on the Buffer Capacity of Activated Saliva.— When activated saliva is left at room temperature, the buffer capacity increases. Even when placed in a refrigerator, a significant change occurs, according to Dewar.[2] As it is not always convenient to titrate saliva immediately, this phenomenon was investigated with the view to discovering its mechanism and a way to preserve samples for buffer capacity measurement. Collection of activated saliva in a test tube with a seal of liquid paraffin and storage of the tube at −18° C. was found to be a satisfactory way to preserve it for this test. The results of a series of tests on frozen saliva will be published in another report. Immediate chilling of saliva in ice also prevents an increase in buffering power, and the change noted by Dewar on refrigeration (4° C.) of samples probably occurred during the time taken by the sample to reach 4° C.

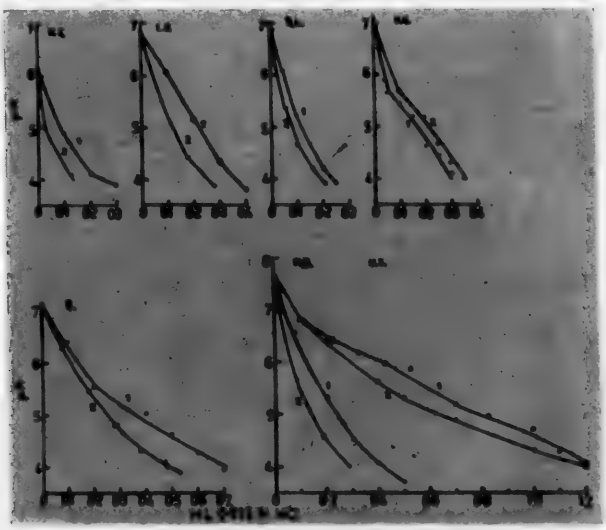


Fig. 9.—Titration curves for salivary sediment. The sediment from 10 ml. saliva was washed once with distilled water and resuspended in 3.0 ml. distilled water. Curve 1: titration under liquid paraffin seal; Curve 2: titration after removal of the bicarbonate system. N.B. The quantity of bicarbonate is represented in each case by the difference between Curve 1 and Curve 2 for the pH range initial value to pH 5.0.

In Fig. 10 are shown typical curves for samples placed in the refrigerator, *A*, from room temperature and, *B*, after immediate chilling in ice. It can be seen that in each case the phosphate buffer system has not changed after 20 hours, whereas in *A* the bicarbonate concentration has increased. On incubation at 37° C. the buffer capacity increases, largely because of increases in (a) bicarbonate and (b) amines, and to a very small extent because of ammonia production. The phosphate concentration remained unaltered (Fig. 11).

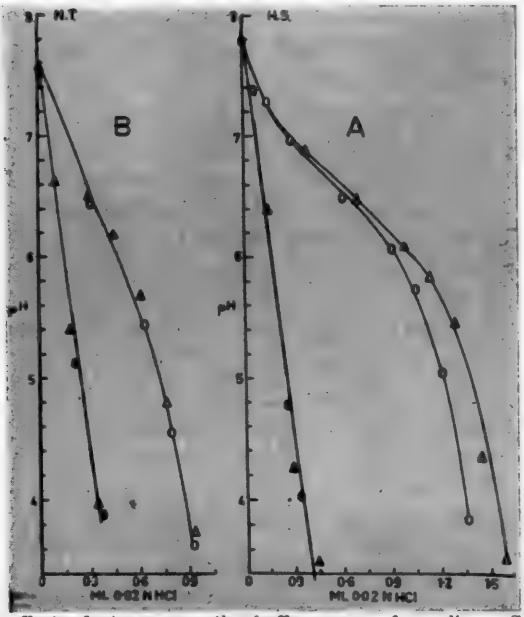


Fig. 10.—The effect of storage on the buffer curves for saliva. Saliva was placed in the refrigerator for 20 hr., *A*: from room temperature, and *B*: after immediate chilling in ice. Titration curves—freshly collected saliva: ○——○ before and ●——● after carbon dioxide removal; stored at 4° C. for 20 hr.: △——△ before and ▲——▲ after carbon dioxide removal.

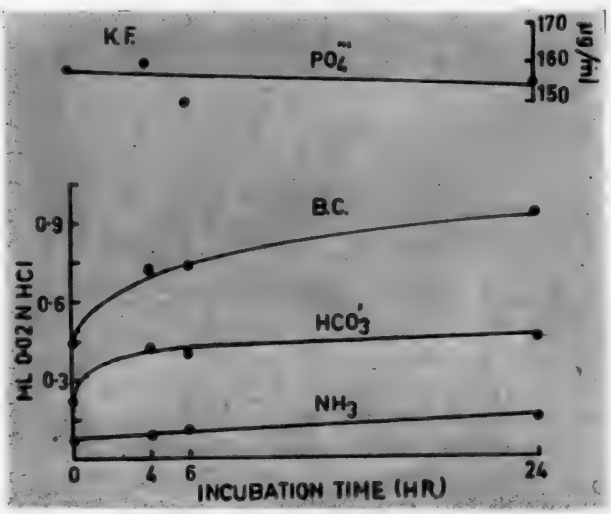


Fig. 11.—The effect of incubation on the buffer systems in saliva. Activated saliva was incubated in a water bath at 37° C. Buffer capacity (B. C.) and other values are for 1 ml. saliva for the pH range initial value to pH 5.0.

The Buffer Capacity in Resting Saliva.—

A. *The role of bicarbonate:* In resting saliva, as shown already with activated saliva, the bicarbonate system plays a major role in the buffer capacity. When the buffer capacity is plotted against that found after removal of the bicarbonate system, it is seen that a considerable variation occurs (Fig. 12).

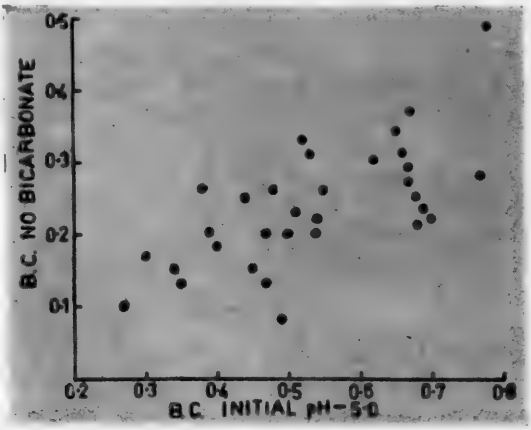


Fig. 12.—Relationship between buffer capacity of resting saliva before and after bicarbonate removal. Values for buffer capacity (B. C.) are expressed in ml. 0.02 N HCl per ml. saliva required to change the pH from the initial value to pH 5.0.

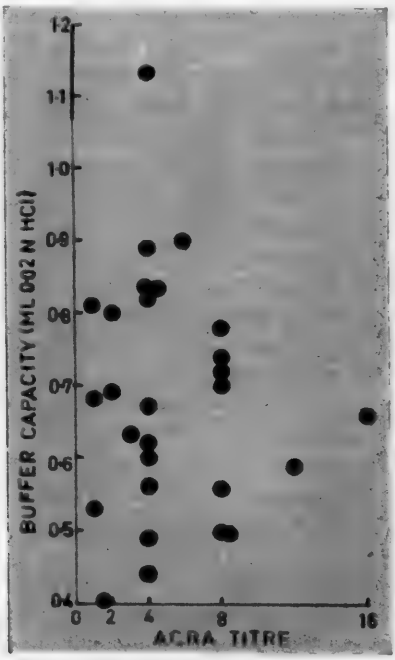


Fig. 13.—The relationship between mucoid content and buffer capacity of resting saliva.

The proportion of the total buffering power which is contributed by the bicarbonate system varies from 30 to 85 per cent. Furthermore, in 85 per cent of the 32 samples studied, this system represented 40 to 70 per cent of the buffering power, i.e., the amount of acid required to change the pH from the initial value to pH 5.0. This is a different situation from that found for activated saliva, where the percentages of the 36 samples investigated showed 64 to 84 per cent of the buffer capacity to be due to the bicarbonate system.

B. *Comparison of mucoid content with buffer capacity:* For the range of pH initial value to 4.0, the buffer capacity was compared with the mucoid content represented by the ACRA titer. This comparison is shown in Fig. 13, which indicates that for each ACRA titer up to 8 considerable differences were found for the buffer capacity. It, therefore, appears that the mucoid content of resting saliva plays no significant part in its buffer mechanism. This is yet another indication of the lack of buffering power of salivary mucoid. In this instance there is least likelihood that the mucoid was changed by the procedures employed.

DISCUSSION

From studies of the carbon dioxide-combining power of the saliva of 11 subjects, Wah Leung[11] presented evidence to show that bicarbonate is the most important buffer in saliva. His results indicated that bicarbonate accounted for approximately 85 per cent of the buffer capacity over the range pH 7.2 to 6.8. In these experiments the estimations were completed within 12 hours of collection of the samples of saliva. Unless saliva is rapidly chilled its buffer capacity increases and, even if the sample is placed in the refrigerator after collection, its temperature does not reach 4° C. for some time, during which period changes occur. The experiments reported here were carried out immediately after the saliva was collected, so that changes in buffer capacity were minimal and the saliva used in the 2 methods for the estimation of carbon dioxide was probably unchanged. This view is based on the observation that estimations of carbon dioxide by the Van Slyke method were the same during this 20-minute period. The results of these investigations give more information concerning the various buffer systems of saliva. Wah Leung[11] merely mentioned bicarbonate buffer and other buffers and suggested that phosphate and proteins made up the remaining buffering activity, particularly at lower pH ranges than 7.2 to 6.8. Again the importance of bicarbonate as a salivary buffer is established and its significance in the buffer capacity test for caries susceptibility is now clear: bicarbonate is the most important buffer present in the saliva collected for this test. Furthermore, the only other buffer system having appreciable activity is probably phosphate. This is indicated by the reduction of buffer capacity on dialysis and complete loss of this smaller capacity when the remaining bicarbonate is removed. Mucoids and proteins must, therefore, have no detectable buffering action in the pH range 8.0 to 4.0.

Removal of bicarbonate by carbon dioxide-free air at a pH below 5.0 satisfactorily disposed of the bicarbonate-carbonate system, as shown by the correlation of the Van Slyke "bicarbonate" estimations with those determined from the titrimetric data.

Direct examination for buffering action of mucoid, precipitated from saliva, failed to confirm the previous claim in this regard (Knox and Still[6]). It would appear that mucoid dissolved in 0.5 per cent Na_2CO_3 and neutralized with HCl was used previously for buffer tests and no allowance was made for the appreciable buffering action of sodium carbonate. Titration of mucoid dissolved in sodium carbonate alone, or added to a phosphate-bicarbonate mixture, showed buffering power due only to the carbonate or phosphate and bicarbonate components in the system. Likewise, precipitated and washed mucoid when added to solutions of HCl failed to alter the pH significantly during observations carried out for several days. There was no correlation between mucoid content of resting saliva and the buffer capacity. Mucoid, therefore, is not a buffer and may merely act as a mechanical barrier to acids and as a lubricant for the oral tissues.

The so-called mucinous plaque (dentobacterial plaque) has been reported to be an excellent buffer (Stralfors[10] and Fosdick[5]). Unfortunately, a clear definition of what is meant by this term "plaque" has not always been given in the reports claiming its buffering power. As mucoid does not act as a buffer, any mucoid in a plaque would not explain its buffering action. The adsorption of bicarbonate, and possibly phosphate, to the bacterial mass in the plaque does not appear to occur. However, salivary sediment when washed has a buffering power, part of which is due to bicarbonate but not to phosphate. Any comparison of the buffering capacity of plaque and saliva, even on a weight basis, does not really give any useful information in connection with neutralization of acids produced on the tooth surface. Obviously the important factors are the rate of acid production by the complex bacterial population adherent to the tooth surface and the rate of its neutralization by the buffers present in close proximity, and the availability of salivary buffers, particularly in regard to both quantity (rate of diffusion through plaques from the outside) and quality. Another factor to be considered under the heading of acid neutralization is the ability of complex mixtures of organisms to destroy acids, e.g., lactic acid, by rapidly metabolizing them. In the curves given by Stephan[9] for pH recovery after glucose rinses, it is obvious that the caries-susceptible plaques have a greater ability to destroy acid than the "immune" ones. The pH scale is a logarithmic one, and the important difference stressed by Stephan[9] was the rate of acid formation. However, the rate of acid destruction or neutralization by the plaques of caries susceptibles is noteworthy.

The process of increase in buffering power on incubation of saliva is one in which bicarbonate and probably amines are formed. Decarboxylation of the amino acids in saliva would give rise to increased bicarbonate and also to amines. The odor of incubated samples supports the contention of amine formation.

In resting saliva, bicarbonate is also the important buffer system and, together with phosphate, probably accounts for all the buffering properties.

SUMMARY

1. Activated saliva owes its buffer capacity largely to the presence of bicarbonate. Phosphate is probably the only other buffer of significance. Al-

though the microorganisms act as buffers, they represent an insignificant part of the buffer mechanism. Mucoid plays no detectable role as a buffer.

2. In resting saliva, bicarbonate and phosphate also represent the buffer system; mucoid was shown to have no influence on the buffer capacity.

3. The buffering mechanisms of salivary sediment and plaque were investigated; the former has adsorbed a variable amount of bicarbonate but the plaque relies only on the organisms for its buffering action.

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EXPERIMENTAL DENTAL CARIES

VI. The Effect of Hypophysectomy on Dental Caries and the Salivary Glands of the Rat

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IT HAS been a constant finding that gonadectomy reduces the caries experience in immature rats[1, 2] and hamsters,[3] but the effect of ovariectomy is less clearly understood. Histologic changes in the salivary glands have been found in gonadectomized animals,[4, 5] thus indicating some relationship between castration and these tissues. Further, the administration of thiouracil has resulted in an increase in dental caries, while desiccated thyroid produced a reduction in dental caries in the rat.[6] It appears, then, that many endocrine dysfunctions modify the caries process in the rat, and since the pituitary gland is of such paramount importance in controlling the over-all functions of the endocrine system, it was considered of importance to learn what effect hypophysectomy had on the dental caries incidence in rats.

EXPERIMENTAL

For this study, 40 albino rats (20 males, 20 females) of the Sprague-Dawley strain were hypophysectomized at 44 days of age while 45 unoperated littermate animals (22 males, 23 females) served as controls. The animals were housed in raised screen-bottomed cages with the cariogenic diet[7] (fluorine content 1.3 μg per gram) and fluorine-low (F = 0.09 μg per milliliter) drinking water available ad libitum. At the termination of the experiment, 137 days after operation, all animals were sacrificed by chloroform inhalation, the heads removed for caries evaluation,[7, 8] the femurs removed for fluorine, calcium, and phosphorus analysis, and various organs taken for microscopic examination.

In an attempt to explain further the relationship between the salivary gland tissues and caries experience, both the amylolytic and the proteolytic activity of salivary gland extracts from these animals were measured.[9-11] The proteolytic activity was determined by surgical removal of the combined submaxillary and sublingual glands, placing the glands from one side of the animal in a lyophilizer, and freeze-drying the tissue until ready for analysis. A stock solution of casein was made by suspending 1 Gm. of Hammarsten casein in 100 ml. of 0.1 M phosphate buffer, pH 7.6. One milliliter samples of the casein substrate were mixed with an equal volume of the previously homogenized salivary gland and heated at 37° C. for 35 minutes. Trichloroacetic acid (5 per cent) was added, and after standing at 25° C. for 1 to 2

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TABLE I

THE EFFECT OF HYPOPHYSECTOMY ON THE DENTAL CARIES INCIDENCE IN RATS, AND LEVEL OF FLUORINE, CALCIUM, AND PHOSPHORUS IN THE SKELETON

GROUP	NUMBER OF ANIMALS	AVERAGE NUMBER OF CARIOUS LESIONS	EXTENT OF CARIOUS LESIONS	SEX	SKELETAL ANALYSIS (ASHED FEMUR)				
					WEIGHT (GM.)	F CONC. (μG/GM.)	TOTAL F (MG.)	CA (MG. %)	TOTAL P (MG.)
Hypophysectomy	39	9.3 ± 0.44*	2.5	M	0.1229	210.80	0.026	37.7	13.8
				F	0.1251	241.50	0.031	37.5	13.9
Control	45	7.6 ± 0.61	1.8	M	0.3643	86.86	0.031	39.2	14.0
				F	0.2831	141.60	0.040	38.8	14.4

*Standard deviation.

hours the precipitate was centrifuged. One milliliter of the supernatant, 1 ml. of 0.0025 M copper sulfate solution, 8 ml. of 0.5 M sodium hydroxide solution, and 3 ml. of dilute Folin-Ciocalteu's phenol reagent were mixed. A similar mixture containing 0.205 mg. tyrosine was prepared, and the color developed in the unknown compared to the tyrosine standard in a Klett photoelectric colorimeter. Computations were based on the concentrations of split glandular products in solution as compared with the tyrosine standard.

The amylolytic activity was determined by quickly weighing the submaxillary gland from the opposite side and macerating it in a mortar for 3 minutes with the use of distilled water which was added at a ratio of 100 ml. per gram of salivary tissue. This homogenate was filtered through gauze and a small aliquot (0.1 to 2.0 ml., depending on anticipated activity as judged from previous studies) transferred to an Erlenmeyer flask (previously warmed to 40° C.) containing 5 ml. of a 1 per cent starch solution, 1 ml. of 0.5 M sodium chloride solution, and phosphate buffer (pH 7.2). The flask and contents were incubated at 40° C. for 15 minutes and 0.5 Gm. of powdered picric acid was added. Following filtration a 3 ml. aliquot was added to 1 ml. of a saturated aqueous sodium carbonate solution. The flask and contents were placed in a boiling water bath for 20 minutes and the solution made to volume and analyzed colorimetrically in a Klett photoelectric colorimeter. A blank and a standard were similarly taken through the entire procedure for comparative purposes.

DATA AND DISCUSSION

The dental caries experiences of the hypophysectomized and intact animals are presented in Table I. The data indicate a significant* difference between the control and operated rats in both the mean number of lesions and the severity of the cavitation. This increase in caries in the operated animals is interesting since it does not correspond to the decrease in caries in male rats brought about by gonadectomy, which has been repeatedly demonstrated. Since hypophysectomy does produce general effects similar to castration, it might be logically assumed that the effect on the dental caries would be similar. However, because these disturbances are much more severe and undoubtedly affect processes not disturbed by gonadectomy, this inconsistency could be explained on this basis. Apparently food consumption played little role in altering the caries experience in this study, for it was noted that throughout the experimental period, the hypophysectomized animals consistently consumed less food than the control animals.

Probably due to the smaller size of the femurs in the hypophysectomized animals, the fluorine concentration was about twice as great as in the unoperated controls. As in previous studies,[14] the females appear to have a higher fluorine concentration than the males, but this may be related to the growth process of the sexes. The total fluorine appears to be somewhat less in the

*As determined by T test. (Snedecor, G. W., *Statistical Methods*, Ames, 1950, Iowa State College Press, p. 76.)

hypophysectomized group, but these differences are so small that their significance is not considered important. No major differences in the calcium or phosphorus content of the skeleton were found in these studies.

The proteolytic and amylolytic activity of the salivary glands from the 2 groups of animals is shown in Table II. The data clearly indicate that the proteolytic activity of the salivary glands of hypophysectomized animals is markedly different from that of the control animals. This corroborates the previous studies of Sreebny[12] and indicates that salivary gland homogenates from hypophysectomized rats show a markedly decreased activity as compared to unoperated controls. Further, the salivary amylase activity appears to be less in the hypophysectomized animals than in the controls. However, as a result of the wide variability, these latter conclusions may be fortuitous. Related studies by Raynaud and Rebeyrotte[13] have shown somewhat similar findings of a reduction of amylolytic activity of the saliva from castrated male mice. These data would seem to indicate that the activity of the salivary gland and the dental caries activity as influenced by hypophysectomy are intimately related.

TABLE II

THE RELATIVE PROTEOLYTIC AND SALIVARY AMYLASE ACTIVITY OF THE SUBMAXILLARY SALIVARY GLANDS FROM CONTROL AND HYPOPHYSECTOMIZED RATS

GROUP	NO. OF ANIMALS	SEX	AV. FINAL WT. OF ANIMALS (GM.)	AV. WT. OF SUBMAXILLARY AND SUBLINGUAL GLAND (1 GLAND) (MG.)	PROTEOLYTIC ACTIVITY		AMYLOLYTIC ACTIVITY	
					RELATIVE ACTIVITY*	ACTIVITY PER GM.†	ACTIVITY‡ (MG./ML.)	RANGE
Control	22	M	330	258	18.7	12.8	6.2	0.35-16.5
	23	F	194	182	15.7	19.2	12.5	3.82-21.8
Hypophysectomy	19	M	113	74	1.3	6.5	3.2	2.36-4.63
	20	F	100	78	1.7	5.6	1.8	0.70-3.7

*Expressed as the relationship between the total gland activity and the activity of the tyrosine standard.

†Expressed as the relationship between gland activity per gram of tissue and the activity of the tyrosine standard.

‡Expressed as mg. of reducing sugar liberated from starch by each milliliter of tissue extract.

Microscopic examination of the salivary glands has shown marked histologic differences between control and hypophysectomized animals. These alterations were of a similar nature in both males and females and were most striking in the submaxillary gland. Any such changes present in the parotid and sublingual glands were of a very minor nature.

The variations in the submaxillary gland were reminiscent of those previously reported in gonadectomized animals,[5] the chief features being a dramatic disappearance of the large granule-bearing tubules in the hypophysectomized rats as well as a peculiar prominent clumping of cells throughout this tissue (Fig. 1). In both the male and female control animals, the large granular tubules were a prominent feature and appeared to be more numerous than the somewhat smaller intralobular ducts. In the operated animals, however,

these granular tubules were uniformly absent, with only the intralobular ducts persisting. In many areas the prominent clumps of cells appeared to be associated with these intralobular ducts and, in some instances, short columns of these cells could be seen leading into the ducts. The cells themselves were small and presented an indefinite outline while exhibiting a scanty cytoplasm with a deeply staining round or ovoid nucleus. The anatomic location of these cells in the secretory unit appeared to be between the intralobular duct and the alveolus, and thus could correspond to the intercalated duct.

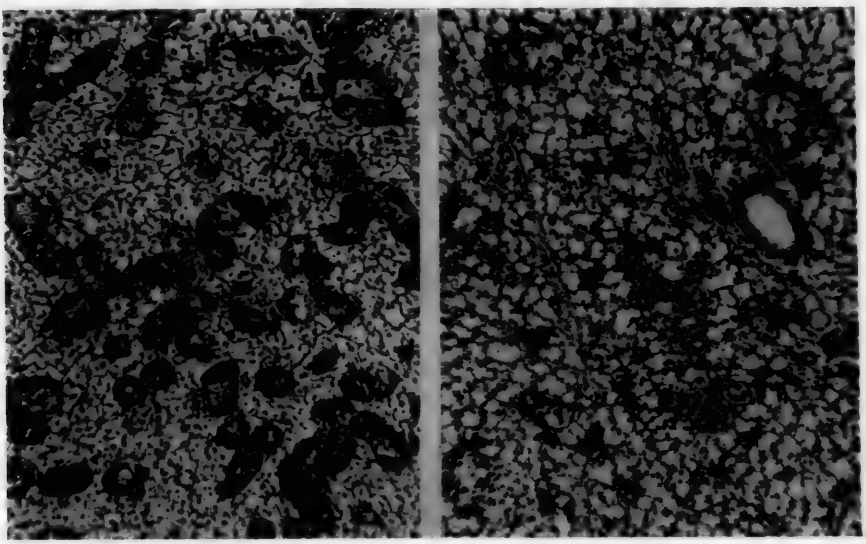

Fig. 1.—Typical hypophysectomized animal on the right exhibits marked changes in the submaxillary gland characterized chiefly by a loss of the granular tubules.

The general histologic alterations noted in the salivary glands of the hypophysectomized animals were similar to those occurring following gonadectomy, administration of diethylstilbestrol or estradiol, and the administration of thiouracil. However, under none of the latter experimental conditions were the changes as severe as in the present study.

The changes in these granular tubules incident to the administration of testosterone, methylandrostenediol, or desiccated thyroid were opposite in nature to those described previously; i.e., instead of a diminution or complete loss of the tubules, an increase in their size and number was noted. Although many studies have been reported dealing in some measure with the structure of these glands, the function of these specific tubules remains to be clarified.

SUMMARY AND CONCLUSIONS

The effect of hypophysectomy on the dental caries experience, as well as on salivary gland structure and function, has been studied in the rat.

There is a significant increase in both the mean number of carious lesions and the severity of the cavitation of the hypophysectomized animals.

There appeared to be a decrease in both proteolytic and amylolytic activity of salivary gland extracts of hypophysectomized animals when compared to control animals.

Marked histologic differences in the submaxillary glands of the two groups of animals were also noted.

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THE EFFECT OF THE CONCENTRATION OF D-GLUCOSE ON THE ATTACK OF TOOTH SUBSTANCES IN THE ARTIFICIAL MOUTH

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THE "artificial mouth" is a device for the reproduction and standardization in vitro of the oral conditions required particularly for studies of the carious process. It is an extension of the numerous earlier studies of in vitro caries that have been carried out since the first experiments of Magitot and Miller.* The device consists of an arrangement for dropping a bacteriologic medium continuously on extracted human teeth, which are inoculated periodically with pooled human saliva.[1] As shown earlier,[2] tooth surfaces cleansed twice daily will remain relatively intact for periods of months; whereas, uncleansed enamel surfaces are rapidly softened and eventually lost. By this procedure, lesions in the enamel of grooves and pits of molars and of the interproximal regions are produced which resemble naturally carious lesions of enamel. A further interesting development of this type is "the experimental mouth."[3]

In the earlier work, however, it was found that, although dentin could be decalcified, the matrix remained relatively resistant to attack. In the present work, conditions are reported which allow destruction of the dentinal matrix as well as permitting decalcification processes. The findings of Burnett and Scherp[4, 5] and earlier workers that intact dentin is relatively resistant to proteolytic microorganisms, whereas decalcified dentin is dissolved, suggested the procedure adopted in the present work.

OPTIMAL CONDITIONS FOR THE ATTACK ON DENTIN AND ENAMEL

Experimental.—The general procedure used in the present work follows the modification described previously.[2] During the experimental period, the temperature was maintained at 35° C. The teeth were brushed only once daily. The basic bacteriologic medium was composed of 0.5 per cent tryptone, 0.2 per cent yeast extract, and 0.025 per cent $CaHPO_4 \cdot 2H_2O$ (sat.) at pH 6.4. Various amounts of D-glucose were incorporated, and in some experiments 0.010 per cent of sodium thioglycollate was present.

All extracted teeth were kept in isotonic saline solution at 4° C. until used. They were then mounted in dental stone in plastic boxes as described earlier,[1, 2] and the surface of the mounting medium was finished with cold-curing acrylic resin.

These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and the Dental School of the University of Alabama (NR 180-017). Some of this work was submitted by Mr. Hawkins as partial requirements for the M.S. degree in the Department of Biochemistry.

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*A survey of the earlier work is given in the earlier publications on the "artificial mouth."[1, 2]

In some experiments, it was desired to expose a section of sound dentin and to expose a section of decalcified dentin. For anterior teeth, this was accomplished by cutting off several millimeters from the incisal edge of a pair of identical anterior teeth. For posterior teeth, the cusps were ground off until dentin was exposed. One area of a posterior tooth, or one tooth of a pair of identical anterior teeth, was covered with low-melting wax. The uncovered portion was then exposed for about 14 days to a continuously flowing sodium lactate buffer. This buffer was 0.081 M in lactic acid and 0.113 M

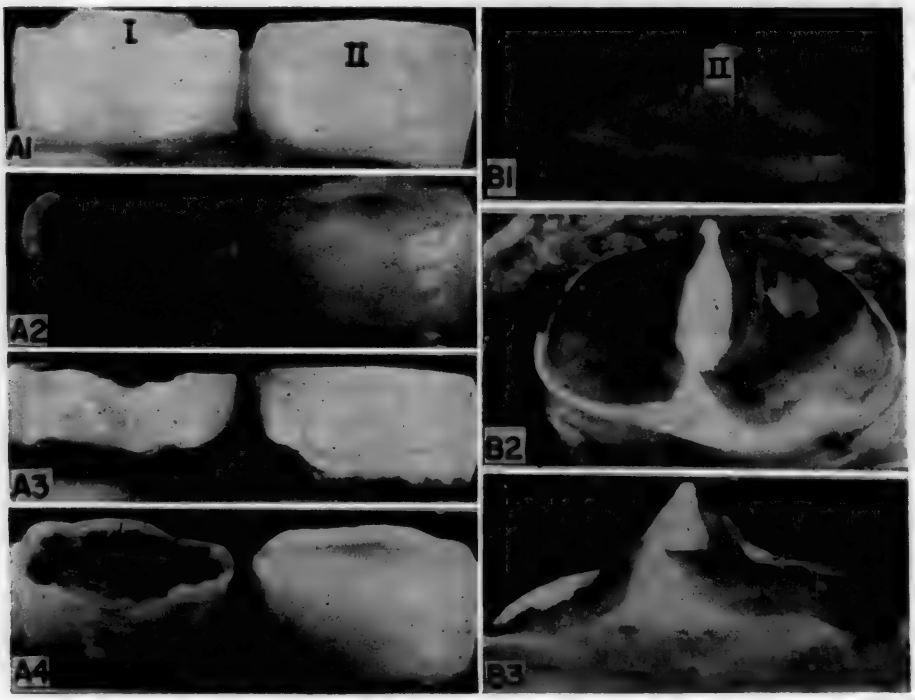

Fig. 1.—Attack on decalcified dentin in the artificial mouth. A pair of cuspids and an impacted third molar exposed to 0.0 per cent glucose and 0.125 per cent glucose, respectively (with tryptone, yeast extract, and $CaHPO_4$).

I, Intact dentin; *II*, decalcified dentin.

A1, Original condition. *A2*, *A3*, *A4*, Condition after 3, 5, and 7 weeks. (Tooth Nos. 6 and 7)

B1, *B2*, *B3*, Condition after 3, 5, and 7 weeks. (Tooth No. 1)

in sodium lactate, pH 4.0. Any remaining loose fragments of decomposed enamel were then removed by brushing. The wax was cut away from the other tooth, and all areas were washed with ether. These stubs of declacified dentin, usually accompanied by a sound tooth or tooth section, are shown in Figs. 1 to 3. Under exposure conditions which definitely brought out the intact dentin these stubs could not be seen on x-ray films.

Results.—The influence of the amount of glucose in the basic medium on the type and rate of attack of sound enamel, sound dentin, and on previously

decalcified dentin is demonstrated in Figs. 1 and 2. In these illustrations, the upper photographs represent the original condition; the teeth have sound sections of enamel and dentin, and portions of dentin decalcified previously by the action of a buffer at pH 4.0.

For the experiments shown in Fig. 1, the glucose concentrations in the media were 0.0 per cent (A1-A4) and 0.125 per cent (B1-B3). At these concentrations of glucose, little or no effect on sound enamel or dentin was noticed. However, the decalcified dentin was markedly softened and destroyed after 3 to 5 weeks.

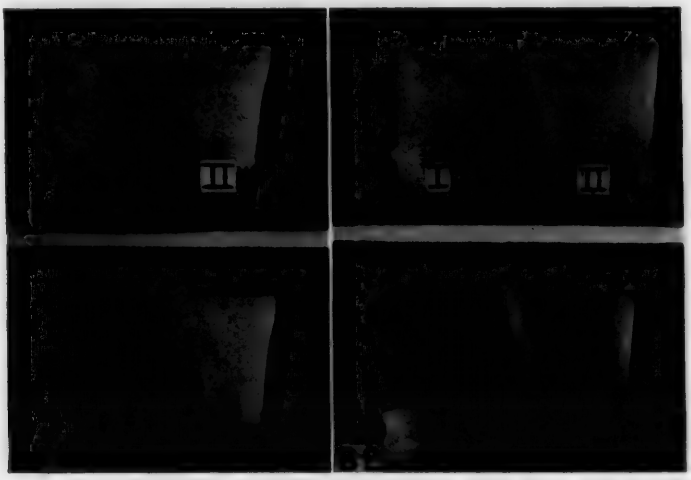

Fig. 2.—Attack on decalcified dentin in the artificial mouth. Lower central incisors exposed to medium composed of 0.25 per cent or 0.5 per cent glucose (with tryptone, yeast extract, and $CaHPO_4$).

A1 and *B1*, Original conditions. *I*, Decalcified dentin; *II*, intact.

A2, Condition after 7 weeks. (Tooth Nos. 13, 14)

B2, Condition after 5 weeks. (Tooth Nos. 23, 24)

For the experiments on which Fig. 2, *A* is based, the glucose concentration was 0.25 per cent. Both enamel and previously decalcified dentin were attacked at the 0.25 per cent concentration. A slight loss of originally sound dentin at the cut incisal edge was also noted after several weeks.

As shown in Fig. 2, *B*, when the glucose concentration was 0.5 per cent, previously decalcified dentin was only slightly affected. Enamel attack was apparent on the cut edges but was only slight on the intact smooth surfaces. It has been noted previously[2] that incisors which have been erupted for a long period of time are quite resistant under these conditions, but that, with most other teeth, enamel is rapidly attacked at this glucose concentration.

A number of experiments were carried out to demonstrate whether attack of decalcified dentin could be facilitated by somewhat anaerobic conditions, such as would be produced by the incorporation of 0.01 per cent sodium thioglycollate in the medium. These experiments were carried out in the same manner as those of Figs. 1 and 2, for the same 4 glucose concentrations

(0.0, 0.125, 0.25, and 0.5 per cent). The results were very similar to those found in the absence of the thioglycollate. An example of these results is given in Fig. 3. Previously decalcified dentin was rapidly destroyed at the 0.25 per cent glucose concentration (Fig. 3, *A*), and enamel attack was the main feature at the 0.50 per cent concentration (Fig. 3, *B*).

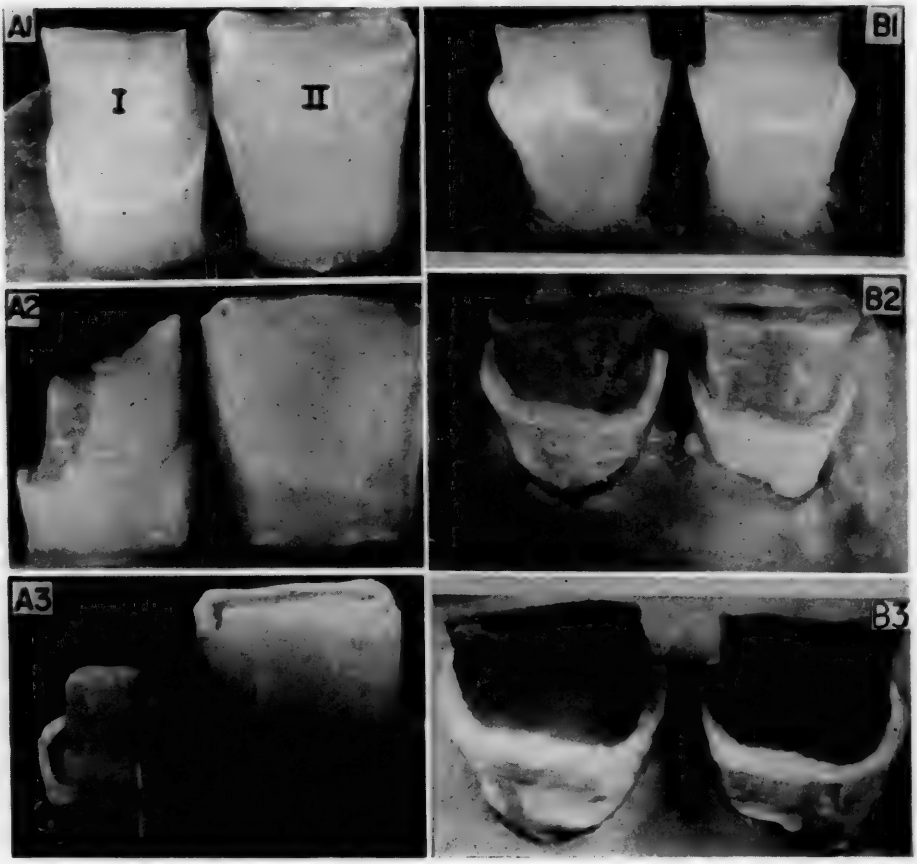

Fig. 3.—Attack on decalcified dentin in the artificial mouth.

A, Lower left central and lateral incisor exposed to medium containing 0.25 per cent glucose and 0.01 per cent sodium thioglycollate (with tryptone, yeast extract, and $CaHPO_4$). (Tooth Nos. 8, 9)

A1, Original condition. *I*, Decalcified dentin; *II*, intact.

A2 and *A3*, After 6 and 8 weeks. Note dentinal destruction and enamel attack in the cervical region.

B, Lower central incisors exposed to medium consisting of 0.5 per cent glucose and 0.01 per cent sodium thioglycollate (with tryptone, yeast extract, and $CaHPO_4$). (Tooth Nos. 2, 3)

B1, Original condition. Exposed dentin was decalcified in both teeth.

B2 and *B3*, After 3 and 7 weeks. Note marked enamel attack.

The results shown in Figs. 1, 2, and 3 indicate that there is a range of glucose concentration around 0.25 per cent at which decalcification of enamel and intact dentin would occur at a rate comparable to the destruction of the decalcified dentinal matrix. Experiments carried out at concentrations of

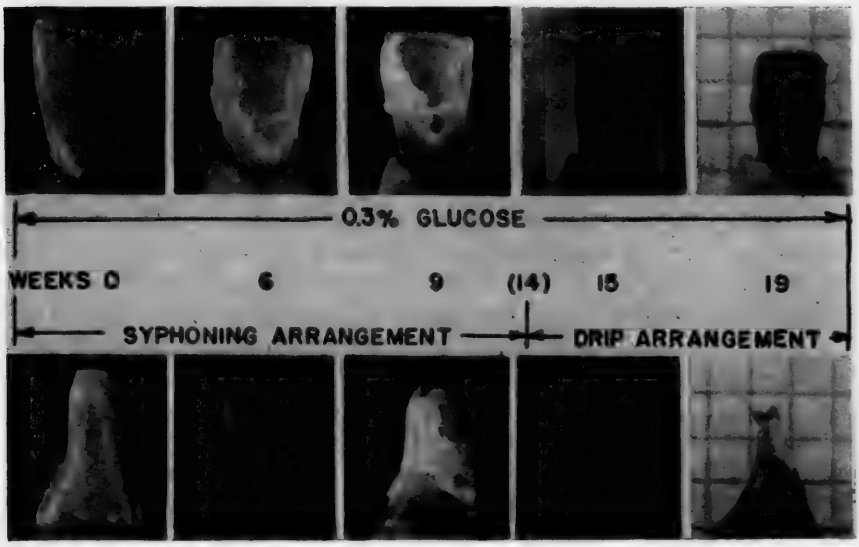

Fig. 4.—Attack on a sound central incisor, without brushing, and with 0.3 per cent glucose in the basic medium. Proximal and lingual surfaces are shown over a period of 19 weeks. (Tooth No. 22)

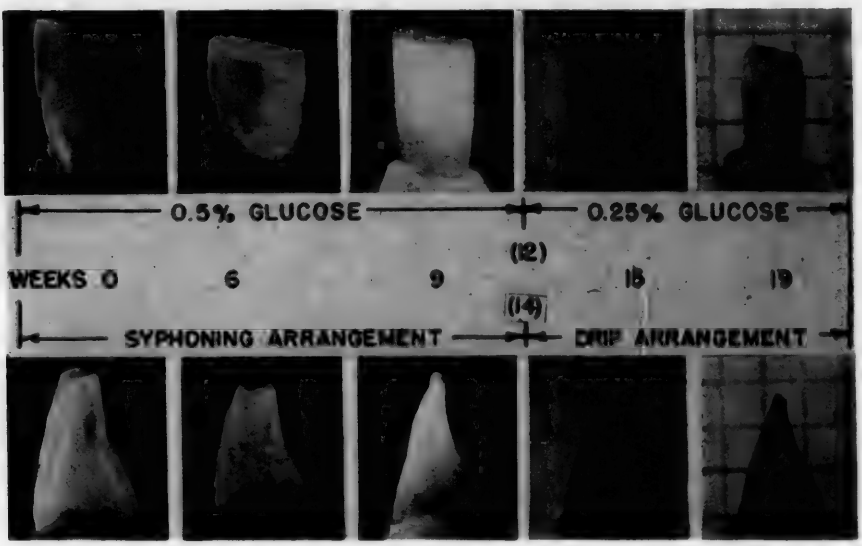

Fig. 5.—Attack on a sound lateral incisor, without brushing. Initial period with 0.5 per cent glucose in the basic medium followed by a decrease in glucose concentration to 0.25 per cent. Proximal and lingual surfaces are shown over a period of 19 weeks. (Tooth No. 21)

0.25 per cent, 0.30 per cent, and 0.35 per cent indicated that this optimal concentration for maximum combined attack was probably somewhat less than 0.25 per cent.

In order to determine whether intact teeth could be extensively destroyed, two experiments were carried out, with the results shown in Figs. 4 and 5. In one of these (Fig. 4, *A*), a lower first incisor was exposed continuously to a medium containing 0.3 per cent glucose with only occasional cleansing and with weekly salivary inoculations. As shown in Fig. 4, the entire enamel surface rapidly softened and almost disappeared within 6 weeks. The exposed dentin was gradually decalcified. This decalcification was accompanied by a brown discoloration, a loss of the surface strength of the decalcified dentin, and eventually destruction of the dentin matrix in some areas.

For another experiment, a second incisor was exposed similarly for 12 weeks to a medium containing 0.5 per cent glucose, and subsequently for 7 weeks to a medium containing 0.25 per cent glucose. During the first period, decalcification and complete loss of the enamel occurred. In the second period, attack of the underlying dentin occurred (Fig. 5). During the second period, all of the exposed dentin developed a yellow-brown color, and the areas of active attack were markedly pigmented.

INFLUENCE OF THE GLUCOSE CONCENTRATION ON THE BACTERIOLOGIC POPULATION

Experimental.—Anterior human teeth were mounted individually in a manner similar to that described previously. However, the cementoenamel junction was about three-eighths inch above the top of the box, and the surface of the mounting medium sloped at an angle of about 60 degrees. The mounted teeth were sterilized for 15 minutes, at 15 pounds pressure. They were inoculated by soaking the crowns for 30 minutes in a sample of pooled saliva from 3 to 5 individuals, for which counts of the lactobacilli and streptococci were made. (Similar inoculations and counts were made weekly on the inoculum.)

The mounted inoculated teeth were placed in the artificial mouth and treated in the usual manner. The medium dropped on the teeth contained 0.5 per cent tryptone, 0.2 per cent yeast extract, and 0.025 per cent $CaHPO_4 \cdot 2H_2O$ at pH 6.4. Variable amounts of D-glucose were also incorporated into the media. The teeth were brushed twice daily with commercial toothbrushes, a separate brush being used for each tooth. The temperature was held constant at 35° C.

Twice each week, the following method was used to estimate the relative number of lactobacilli and streptococci on the cleansed surfaces: the entire crown was brushed under running tap water for at least one minute with a commercial toothbrush; the tooth was allowed to dry for one minute, and the surfaces of the box and mounting material were wiped dry with a cloth; the box was then placed in an inverted position over the opening of a cylindrical glass vial containing 34 (±1) c.c. of similar sterile medium having 0.5 per cent glucose, but without dicalcium phosphate; only the crown was in

contact with the medium, and special efforts were made for each tooth always to expose the same amount of crown; the tooth was gently rotated about 5 times to remove air bubbles and to ensure good contact with the medium; the crown was kept in contact with the medium for 30 minutes. Finally, the tooth was again rotated gently several times and removed. The bottle was capped and shaken mechanically for 5 minutes. Samples of these solutions were diluted 1:1,000 with sterile distilled water and 1 c.c. portions were plated out in selective lactobacillus agar (BBL) and Difco brain-heart infusion agar containing sodium azide and crystal violet. The plates were allowed to solidify, and after 72 hours of incubation, colony counts were made on duplicate plates by 2 individuals. Saliva was treated similarly except for the dilution which was 1:1,000 or 1:10,000 for the lactobacilli and 1:10,000 and 1:100,000 for the streptococci.

Results.—The data given in Table I show that streptococci were present at all the glucose concentrations that were studied, but that there was very little increase in relative amounts above the 0.25 per cent concentration. In contrast, no lactobacilli could be demonstrated at glucose concentrations below 0.25 per cent, and at this concentration, the relative counts were low. At concentrations of 0.375 and 0.5 per cent, the numbers of lactobacilli increased and were of the order of magnitude of the streptococcus counts.

TABLE I

EFFECT OF GLUCOSE CONCENTRATION ON THE RELATIVE NUMBER OF LACTOBACILLI AND STREPTOCOCCI ON TOOTH SURFACES*

	0.00 PER CENT GLUCOSE		0.125 PER CENT		0.25 PER CENT		0.375 PER CENT		0.5 PER CENT		SALIVA USED FOR INOCULATION	
DAYS	L	S	L	S	L	S	L	S	L	S	L	S
0	–	–	–	–	–	–	–	–	–	–	250	3,500
4	0	4	0	0	2	8	2	98	4	20		
8	0	2	0	0	1	2	8	41	11	20	350	7,000
11	0	4	0	3	0	61	42	33	6	59		
15	0	4	0	29	2	176	111	153	10	24	1,000	4,000
18	0	10	0	6	3	33	86	111	8	20		
23	0	22	0	1	1	3	8	14	8	13	600	2,500
26	0	21	0	17	0	14	11	43	20	14		
30	0	21	0	7	1	52	2	12	14	5	200	500
33	0	4	0	7	2	37	26	15	31	5		
37	0	4	0	6	4	25	8	8	12	4	25	1,000
40	0	4	0	4	2	36	17	28	40	16		

*The media were composed of the indicated amount of D-glucose with 0.5 per cent tryptone, 0.2 per cent yeast extract, and 0.025 per cent $CaHPO_4 \cdot 2H_2O$.

The values given are colony counts from 1 c.c. of 1:1,000 diluted media in which the crowns of the teeth had been immersed. *L*, Lactobacillus counts; *S*, streptococcus counts.

In some earlier experiments, it appeared that at the 0.375 and 0.5 per cent glucose concentrations, the lactobacillus counts gradually increased with time, whereas the streptococcus counts decreased. As shown in Table I, however, these results could not be reproduced, and there seems to be no definite replacement of one organism by the other.

QUALITATIVE STUDY OF THE MICROBIAL POPULATION AS A FUNCTION OF THE COMPOSITION OF THE MEDIUM

A preliminary study was made of the organisms which could be cultured from the scrapings of several of the teeth used in the experiments to demonstrate attack of enamel and of decalcified dentin. A small amount of surface material was removed with a dental probe and placed in 10 c.c. of sterile, distilled water. After the solution had been shaken about 100 times, 1 c.c. aliquots were placed in Petri dishes. To these were added brain-heart infusion medium (Difco) with added agar (BHI) at pH 7.2, or with added 0.02 per cent sodium azide. Another 0.5 c.c. was placed in fluid Brewer's thioglycollate broth (BTB). Another aliquot was placed in BBL lactobacillus selection medium, prepared according to Rogosa (LSM). Examinations at 24 or 48 hours were made.

The qualitative identifications were made on scrapings from teeth exposed to the standard medium containing 0.01 per cent sodium thioglycollate, and with the following amounts of added D-glucose: 0.0 per cent, 0.25 per cent, and 0.5 per cent. In the absence of added glucose, no lactobacilli could be isolated. Motile gram-negative rods (probably-*Pseudomonas fluorescens*), *Micrococcus pyogenes* var. *aureus*, and a gamma-hemolytic streptococcus were found, however. In scrapings from teeth exposed to the medium having a glucose concentration of 0.25 per cent, the following organisms were isolated: a gram-negative rod of the proteus type, a gram-negative diplococcus from a dry colony (probably *Neisseria sicca*), and *Micrococcus pyogenes* var. *aureus*. From teeth exposed to the 0.5 per cent glucose concentration, motile gram-negative rods (probably *Pseudomonas fluorescens*), gram-positive short rods, *Micrococcus pyogenes* var. *aureus*, and lactobacilli sp. were isolated.

DISCUSSION

The present work is in close agreement with the previous observations* of Miller, Giles, Goadby, McIntosh, Bibby, Burnett and Scherp, and others that intact tooth substance is quite resistant to destruction by bacteria unless prior decalcification has taken place, and that decalcified dentin is destroyed by oral microorganisms. Weisberger[6] also noted earlier that extracted teeth remained unchanged to visual examination in media which supported the growth of mixed oral microorganisms, whereas enamel attack was pronounced when 1 per cent glucose was added. In the present work, however, the conditions more closely approximate oral physiologic conditions, and a marked effect of glucose concentration has been demonstrated. Decalcification becomes dominant in the higher concentrations of glucose. At low glucose concentrations, intact tooth substances, enamel, and dentin (and probably cementum) are quite resistant, but decalcified dentin is rapidly softened, discolored, and destroyed. The two ranges overlap, and at a concentration of about 0.25 per cent glucose, intact enamel and dentin are attacked fairly rapidly, by both decalcification and destruction of the matrix.

*References to this earlier work are given by Burnett and Scherp.[4, 5]

In agreement with the earlier work, it is again shown that the processes of decalcification, and destruction of the organic matrix, can be independent processes, and that decalcification must precede at least slightly the attack on the organic matrix. As shown in the previous paper[2] of this series, however, complete enamel loss can occur by purely decalcification procedures, and there is no evidence that the enamel protein requires degradation.

Burnett and Scherp[4, 5] found that the only known oral microorganism which would rapidly decalcify intact dentin was lactobacillus. It is of considerable interest, but probably coincidental, that in the present work decalcification became noticeable at the concentration of glucose (0.25 per cent and greater) at which the presence of lactobacilli could be demonstrated. Attack of decalcified dentin, however, was greatest at concentrations less than 0.25 per cent glucose, at which no lactobacilli could be detected.

SUMMARY

Under the "standard" conditions for the operation of the artificial mouth, it is demonstrated that the attack at relatively high glucose concentrations (0.5 per cent) is mainly decalcification, whereas at glucose concentrations of 0.25 per cent and less, previously decalcified dentin is softened, pigmented, and, finally, completely destroyed. When the glucose concentration is about 0.25 per cent, both decalcification and dentinal matrix destruction occur fairly rapidly; the dentin, enamel, and cementum of sound teeth are extensively destroyed in a period of several months.

A method was developed for determining the relative numbers of streptococci and lactobacilli associated with a single tooth. It was shown that the streptococci were present at all the glucose concentrations studied, but seemed to reach a maximum at about 0.25 per cent glucose. Lactobacilli required the presence of a minimum of about 0.25 per cent glucose for survival. A preliminary qualitative study was also made of the organisms found in the plaque when the medium contained 0.01 per cent sodium thioglycollate and variable amounts of glucose.

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INCIDENCE OF DENTAL CARIES, GINGIVITIS, AND MALOCCLUSION IN CHICAGO CHILDREN (14 TO 17 YEARS OF AGE)

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INTRODUCTION

EXTENSIVE studies on the epidemiology of dental diseases, especially dental caries, have been reported in literature. Recently, some attempts have also been made to assess gingival disease as well as malpositioning of the teeth.

A review of the literature revealed that only two reports citing the incidence of all three diseases in the same population had been compiled. Messner, Gafafer, Cady, and Dean[1] reported a dental survey of school children 6 to 14 years of age (data being collected by 8,000 dentists) in 26 states, while Brucker[2-4] reported the incidence of gingivitis, malocclusion, and caries in a series of publications. However, he examined different groups of children for the assessment of each disease.

The availability of epidemiologic methods of assessment known as DMF index[5] for dental caries, P–M–A index for gingivitis,[6] and malocclusion index (counting the number of malocclusion teeth), as advocated by Massler and Frankel,[7] presented the opportunity of studying these three conditions in the same group.

This paper is a report of the incidence of dental caries, gingivitis, and malocclusion in children 14 to 17 years of age. An earlier publication presented the findings on the relation of gingivitis to dental caries and malocclusion of the same group of children.[8]

MATERIALS AND METHODS

This study is based on the examination of 2,774 children of Morton High School, Cicero, Illinois. The sample included an almost equal distribution of boys (1,291) and girls (1,483) (Table I). One hundred and seven children undergoing orthodontic treatment were excluded from the study. A detailed description of the sample of children as well as the methods of examination and assessment has been published earlier (Massler and Savara[8]). Oral examinations were carried out by three separate teams, each consisting of two persons, one team each for caries, gingivitis, and malocclusion. DMF index method as described by Klein, Palmer, and Knutson[5] was used for the assessment of dental caries experience. Gingivitis was assessed and recorded according to the P–M–A method outlined by Massler, Schour, and Chopra.[6] The three figures (P, M, and A) were added together as P+M+A, and the one figure was used

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for expressing total gingivitis. This usage was justified in earlier studies.[8] Malocclusion was determined using Angle's classification and by counting the number of malocclueded teeth, according to the method outlined by Massler and Frankel.[7]

TABLE I

DENTAL CARIES EXPERIENCE (DMF TEETH AND DMF TOOTH SURFACES) IN 2,774 CHILDREN 14 TO 17 YEARS OF AGE

AGE IN YEARS	NUMBER OF CHILDREN EXAMINED			AVERAGE NUMBER OF DMF TEETH PER CHILD			AVERAGE NUMBER OF DMF TOOTH SURFACES PER CHILD		
	BOYS	GIRLS	COMBINED	BOYS	GIRLS	COMBINED	BOYS	GIRLS	COMBINED
14	214	363	577	7.39	9.06	8.4	13.36	19.27	17.0
15	328	400	728	7.97	9.75	8.9	15.10	18.23	16.8
16	340	317	657	9.98	10.77	10.3	19.65	20.34	19.9
17	409	403	812	10.53	11.86	11.2	20.13	22.64	21.4
14 to 17	1,291	1,483	2,774	9.20	10.39	9.83	17.57	20.15	18.95

FINDINGS

A. *Dental Caries Experience.—*

During the course of this survey, it was obvious that almost all of the children had suffered from caries at one time or another. Of the 2,774 children examined (14 to 17 years of age) 98.2 per cent had one or more teeth affected by dental caries. The average amount of dental caries found in the total group was 9.8 DMF teeth (19.0 DMF tooth surfaces) per child (Table I). Dental caries experience increased markedly with age from 14 to 17 years in both boys and girls.

Sex differences: The average dental caries experience in girls (20.1 DMF tooth surfaces and 10.3 DMF teeth) was higher than that of boys (17.5 DMF tooth surfaces and 9.2 DMF teeth). This difference was found consistently at each age level (Table I, Fig. 1). The differences in dental caries experience between boys and girls were analyzed statistically and found to be significant ($P < 0.001$ t value $= 7.433$).

Sex differences in dental caries experience have already been pointed out by Klein and Palmer[9] for children under 15 years of age and by Hollander and Dunning[10] in persons over 17 years of age. The data presented here establish the fact that a significant difference in dental caries experience also exists in boys and girls from 14 to 17 years of age.

Comparison with Hagerstown children: When the caries experience in this group is compared with the caries experience of Hagerstown children as described by Klein, Palmer, and Knutson,[5] it is obvious that the Cicero children represent a much more caries-susceptible group (Fig. 1). The Cicero children showed approximately 1.5 DMF teeth and 3.0 DMF tooth surfaces more than the Hagerstown children at each age level.

Number of carious (DMF) surfaces per carious (DMF) tooth: The ratio between the number of DMF tooth surfaces and DMF teeth defines the extent of the carious involvement on the "average" tooth. In this group, the average

caries involvement per child was 19.0 DMF tooth surfaces or 9.8 DMF teeth (almost 2:1). The ratio of approximately 2 surfaces affected for each carious tooth indicates the fact that the average carious tooth (filled or unfilled) had approximately 2 affected surfaces.

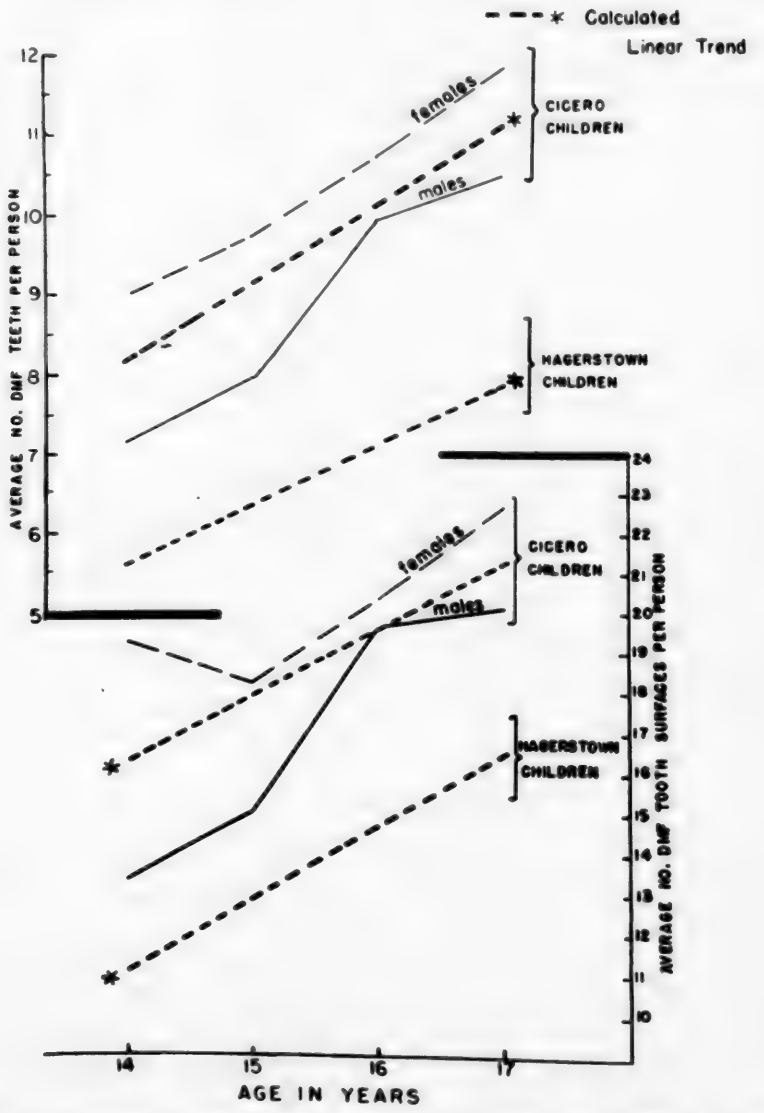

Fig. 1.—Incidence of dental caries (DMF teeth and DMF tooth surfaces) in 2,715 boys and girls 14 to 17 years of age. The difference in caries experience between boys and girls is statistically significant ($t = 7.433$ and P is less than 0.001). The trend lines are drawn for comparison with Hagerstown children.[5] Cicero children show a much higher caries experience than Hagerstown children. Rate of increase (acceleration) in caries experience is the same for Cicero and Hagerstown children and is the same whether measured as DMF teeth (0.82 tooth per year) or DMF tooth surfaces (1.7 tooth surfaces per year).

B. *Gingivitis.*—

Of the 2,774 children examined, 39.3 per cent had one or more of their gingival units (papillary, marginal, or attached gingivae) affected by gingivitis. The average amount of gingivitis was approximately 2.2 papillae and 1.8 margins, or a total of 4.0 P+M+A gingival units, affected per child (Table II, Fig. 2). The attached gingivae were very rarely affected (3 children in the

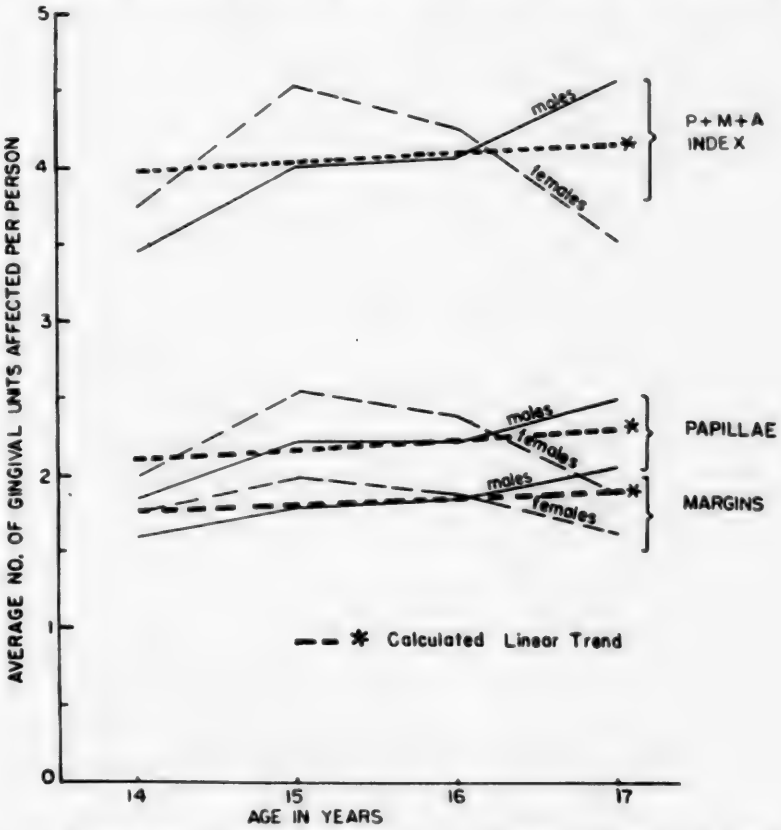

Fig. 2.—Gingivitis experience (papillary and marginal gingivitis and P+M+A index) in 2,774 boys and girls from 14 to 17 years of age (note the parallelism in the linear trend of the 3 ginigival units).

TABLE II

GINGIVITIS EXPERIENCE (P–M–A AND P+M+A) IN 2,774 CHILDREN 14 TO 17 YEARS OF AGE

AGE IN YEARS	AVERAGE NUMBER OF GINGIVAL UNITS AFFECTED PER PERSON											
	NO. PAPILLAE AFFECTED			NO. MARGINS AFFECTED			NO. ATTACHED GINGIVAE AFFECTED			NO. P+M+A AFFECTED		
	BOYS	GIRLS	COMBINED	BOYS	GIRLS	COMBINED	BOYS	GIRLS	COMBINED	BOYS	GIRLS	COMBINED
14	1.84	2.00	1.94	1.59	1.76	1.71	0	0	0	3.46	3.76	3.64
15	2.22	2.55	2.4	1.78	1.98	1.9	0	0	0	4.01	4.54	4.30
16	2.22	2.38	2.3	1.84	1.88	1.86	0	0.018	0.0091	4.07	4.27	4.14
17	2.49	1.90	2.2	2.06	1.62	1.89	0.004	0	0.0029	4.56	3.52	4.08
14 to 17	2.24	2.20	2.23	1.85	1.80	1.84	0.0015	0.004	0.0028	4.09	4.00	4.06

entire group). There did not appear to be any significant increase or decrease in gingivitis experience with increasing age. Linear trends for the incidence of papillary gingivitis and marginal gingivitis were also inspected and compared with the combined value (P+M+A). The parallelism among the 3 values was striking (Fig. 2).

Sex differences: Slight differences in gingivitis experience could be observed between boys and girls but were not statistically significant. Girls from 14 to 16 years of age had a greater number of gingival units affected, but a lesser number at 17 years; the boys showed a slight increase in the incidence of gingivitis with increasing age.

C. *Malocclusion Experience.*—

A complete and detailed analysis of the incidence of malocclusion in this group has been reported separately (Massler and Frankel, 1951[7]). Only 2.9 per cent of the 2,758 children had perfect (ideal) occlusion, and the remainder showed at least one or more teeth out of perfect occlusion (Table III). However, 20.2 per cent of the children had very mild displacement of only a few teeth. Their occlusion was "normal" (although not ideal) in the sense that they were "almost perfect" and did not require treatment for malocclusion. Therefore, 77.2 per cent of the children suffered from a significant degree of malocclusion which under ideal circumstances would require treatment. Distribution of different types of malocclusion according to Angle's classification showed that 50.07 per cent of the children had Class I, 16.68 per cent exhibited Class II, Division 1, 2.71 per cent were with Class II, Division 2, and 9.43 per cent showed Class III type occlusion.

TABLE III

INCIDENCE OF MALOCCLUDED TEETH AND DISTRIBUTION OF CHILDREN ACCORDING TO OCCLUSION

AGE IN YEARS	AVERAGE NUMBER OF TOTAL MALOCCLUDED TEETH PER CHILD			% CHILDREN "IDEAL"* OCCLUSION	% CHILDREN "NORMAL"† OCCLUSION	% CHILDREN WITH MALOCCLUSION‡			
	BOYS	GIRLS	COMBINED			CLASS I	CLASS II DIVISION 1	CLASS II DIVISION 2	CLASS III
14	11.38	10.08	10.56	3.80	17.72	51.17	15.37	2.53	9.40
15	10.57	10.78	10.69	2.61	17.17	50.41	17.73	3.70	8.38
16	10.81	9.45	10.09	2.89	19.34	48.85	16.21	2.71	9.90
17	11.19	10.45	10.81	2.51	18.36	50.06	17.07	1.93	10.01
14 to 17	10.96	10.22	10.55	2.93	18.23	50.07	16.68	2.71	9.43

Data courtesy of Dr. John M. Frankel.

*"Ideal" occlusion was reserved for those cases in which not a single malocclude tooth could be discovered.

†"Normal" occlusion was used to designate those cases in which the number of malocluded teeth was less than the average number (10), and the degree of displacement was so small as to catalogue it clinically as "normal" and requiring no treatment. Some degree of subjectivity was necessary in such cases in order to determine whether they belonged in the category of "normal" or "mild Class I." For the most part, the decisive factor was whether or not orthodontic treatment was or was not indicated.

‡Defined according to Angle's classification.

Incidence of malocclude teeth: The average number of malocclude teeth per person for the total population was 10.5 (Table III). The line of trend (linear regression), as plotted on Fig. 3, revealed no change in malocclusion experience with increasing age. This was true for both sexes and indicates that there is no tendency for the severity of the existing malocclusion to increase

during this period. The flat linear trend can thus be interpreted to indicate that the tendency for physiologic malocclusions to become self-corrected is ended by the fourteenth year. However, these interpretations are based on cross-sectional data and should be checked by longitudinal studies.

Sex differences: Marked differences in the amount or severity of malocclusions between boys and girls were not apparent. The boys showed a higher average number of malocclused teeth than did the females at every age level except 16 years (Table III, Fig. 3). The average number of malocclused teeth in boys of all ages (10.9) was higher than in girls (10.2). This difference between boys and girls when subjected to statistical analysis was found to be insignificant.

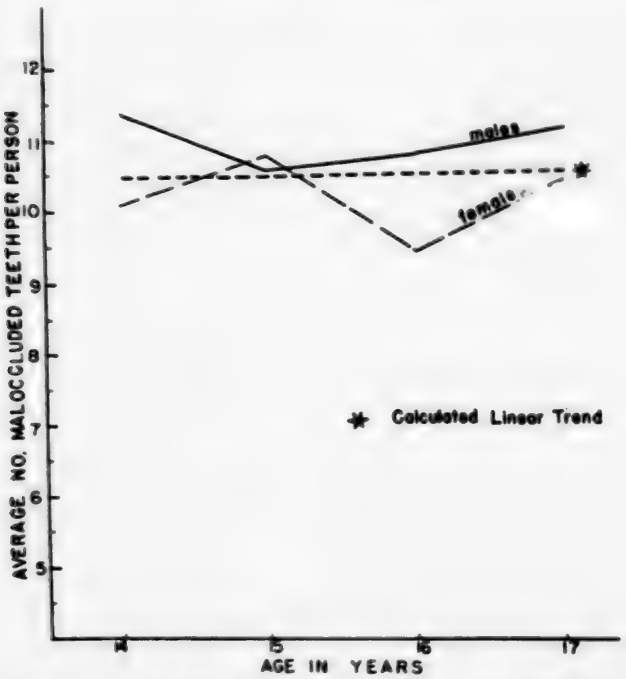

Fig. 3.—Average number of malocclused teeth per person in 2,758 boys and girls from 14 to 17 years of age. Note the flat linear trend indicating no increase in the number of malocclused teeth with age.

Relation of Gingivitis to Dental Caries and Malocclusion.—The relation of gingivitis to caries and malocclusion was studied by plotting scatter diagrams between the number of gingival units (P+M+A) affected to the number of carious (DMF) tooth surfaces in each person and number of gingival units to the number of malocclused teeth in each person. No relationship was found to exist between gingivitis and dental caries and malocclused teeth. Dental caries experience (DMF teeth and DMF tooth surfaces) in children with no gingivitis and children with severe gingivitis was compared and it showed that the children in the severe gingivitis group had more decay. The difference between the two groups was significant ($t = 5.243$, $P < 0.001$). Similarly, a

comparison of gingivitis (P+M+A) in children with low and high dental caries experience revealed that the children in the severe caries group had more gingivitis and the difference was statistically significant ($t = 5.968$, $P < 0.001$).

The malocclusion experience in children with no gingivitis and children with severe gingivitis was studied and found to be statistically different and significant ($t = 7.189$, $P < 0.001$). From these findings, it was concluded that children with severe gingivitis had a tendency to experience higher caries attack rate and had a larger number of malocclueded teeth than did the rest of the children.

SUMMARY

Incidence of gingivitis, dental caries, and malocclusion in 2,774 children 14 to 17 years of age is reported. The average amount of gingivitis was approximately 2.2 papillae, 1.8 margins, or a total of 4.0 P–M–A gingival units, affected per child. There was no change in gingivitis with age. A total of 98.2 per cent of the children had one or more decayed teeth. The average amount of dental caries was 9.8 DMF teeth or 19.0 DMF tooth surfaces. Girls had a higher and statistically significant incidence of dental caries as compared to boys. A total of 77.2 per cent of the children had malocclusion, the average child having 10.5 malocclueded teeth. There was no significant difference in the incidence of malocclusion between boys and girls. The variation in incidence of the 3 diseases between 14 to 17 years of age is discussed.

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OBSERVATIONS ON THE PERMEABILITY OF HUMAN CALCIFIED DENTAL TISSUES TO PENICILLIN

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PENICILLIN and other antibiotics are being used to an increasing extent in root canal therapy, and it is a matter of clinical importance to know whether penicillin diffuses out through the whole of the root surface, part of the root surface, or only through the apical foramen when it is sealed in the pulp cavity. According to Hopewell-Smith,[9] there is at the cementum-dentin junction a narrow structureless zone of dentin which provides an efficient barrier to the passage of medicaments inserted into the pulp cavity. A structureless zone can indeed often be seen in ground sections of the roots of human teeth intervening between the granular layer of Tomes and what is frankly cementum. Some writers, including Hopewell-Smith,[9] have described this zone as dentin, whereas according to others it is a zone of cementum (Mummery,[12] Bencze[4]).

Stewart-Ross[15] found that when methyl blue was introduced into the pulp cavities of extracted teeth of dogs the dye entered the cellular cementum at the apex but, except in very young dogs, the cementum-dentin junction of the remainder of the root was not traversed by the dye. His experiments on monkeys in vivo, involving infection of the pulp cavities with microorganisms and insertion of irritant drugs into pulp cavities, appeared to show that there exists in these animals an impermeable barrier in the region of the cementum-dentin junction. Stones[16] inserted aniline dyes in the pulp cavities of the teeth of young and adult dogs and found that, whereas in very young dogs the dye diffused slowly from the dentin into the cementum, in adult dogs the cementum-dentin junction, except in the region of the apex, had become very resistant to the passage of dyes. In experiments in living dogs and monkeys MacGregor[11] found lead acetate (basic lead acetate, m.w.566.5) in the terminal arborizations of the dentinal tubules beneath the cementum following the introduction of this substance into the gingiva. He stated that there was considerable doubt whether the acetate in the dentinal tubules had reached this situation by passing through the cementum.

The deduction has often been drawn from the experiments of Stewart-Ross and Stones in laboratory animals that, at least in the adult, the cementum-dentin junction of human teeth is likewise impermeable to dyes and molecules of similar size, and to tissue fluid. The commonplace clinical observation that when pulp cavities become the site of a profuse growth of pathogenic bacteria, inflammatory changes in the periodontal membrane are confined as a rule to the region of the apex is significant in this connection. If the cementum-dentin junction and the cementum were permeable to these agents there is little doubt that inflammatory changes in the periodontal membrane would be more widespread.

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Penicillin (sodium benzyl penicillin, m.w.356.4) has a molecular size very approximately similar to those of aniline dyes (methyl green, m.w.458.5; acid fuchsin, m.w.585.5; methyl blue, m.w.799.8; Conn[7]). On the basis of the observations referred to previously, it might be inferred therefore that penicillin inserted into the pulp cavities of human teeth would not diffuse through the cementum and into the periodontal membrane, except perhaps in the region of the apex, where the cementum is of the cellular variety and contains lacunae and canaliculi. Shuttleworth,[13] however, sealed penicillin into the pulp cavities of recently extracted caries-free human teeth with gutta percha and wax. The teeth were then placed on their sides on the surface of agar plates spread with penicillin-sensitive microorganisms. Twenty-four hours later wide areas of inhibition of bacterial growth had developed. From these preliminary experiments and similar ones designed to study the permeability of enamel, Shuttleworth concluded that penicillin inserted into pulp cavities diffuses through the cementum covering the root and that penicillin penetrates the enamel.

The unexpected character of Shuttleworth's finding concerning cementum permeability and the importance of this matter from the clinical point of view led us to undertake the present investigation. Atkinson,[1] in the course of his study of enamel permeability, concluded that an efficient and reliable seal involving teeth could not be obtained with waxes or cements. He found that rubber connections under pressure were necessary to achieve a perfect seal. In the present investigation methods other than wax seals were therefore employed.

MATERIAL

All the teeth used in the following experiments were human permanent teeth, caries-free on naked-eye inspection after extraction. Single-rooted teeth were used except where otherwise stated. In most cases the experimental procedures were commenced either immediately or within 1 hour of the extraction, being kept in saline solution in the meantime. Where the interval was longer the teeth were stored in saline solution in a refrigerator at about 5° C., but in no case was the interval longer than 24 hours.

Sodium benzyl penicillin was the preparation of penicillin employed in the investigation.

EXPERIMENTAL PROCEDURES

(A) *Permeability of the Root.—*

Method 1: The crowns and apices of the teeth were removed with a fret saw. The root canals were enlarged by reaming and the roots were then dipped in absolute alcohol and momentarily flamed in order to dry the surfaces and remove some of the bacterial contaminants. The ends of each root were pushed into sterile rubber tubes of appropriate diameter and secured with an annealed wire ligature (Atkinson[1]), leaving 2 to 4 mm. of the middle part of the root exposed. The open end of one rubber tube was closed by ligaturing around a closed glass tube (Fig. 1), and an open glass tube was similarly ligatured to the other rubber tube. Through the open end of this system penicillin solution (50 units per milliliter) was introduced by means of a fine glass pipette and the

lumen of the system was almost completely filled with the solution. The open end of the glass tube was then sealed with heat. Rigorous precautions were taken to avoid contamination of the outer surfaces with penicillin.

The roots and attached tubes were placed in Petri dishes and just covered with melted nutrient agar. The dishes were then placed in a refrigerator for periods varying from 3 to 72 hours in order to provide time for the penicillin to diffuse. At the end of this time the plates were inoculated with a standard culture of penicillin-sensitive *Staphylococcus pyogenes* (Oxford strain).

In order to provide a control, a disc of the inoculated agar was removed with a cork borer leaving a well or "control window," in which was placed a drop of the same penicillin solution used to fill the prepared tubes. The agar plates were then incubated at 37° C. overnight (12 to 24 hours).

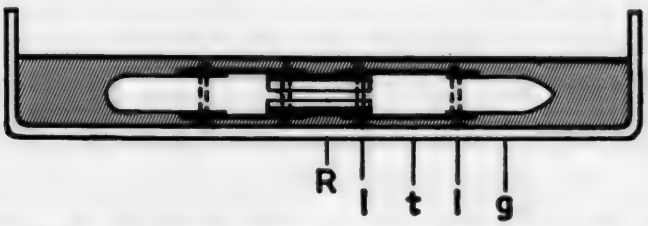


Fig. 1.—Method 1. Root with ligatured rubber and glass tubes, immersed in agar. Approximately 2.4 mm. of the root is exposed. *R*, Root; *l*, wire ligatures; *t*, rubber tubes; *g*, glass tubes.

After the presence or absence of areas of inhibition of bacterial growth was noted, in several cases the tubes were removed and opened. A sample of the penicillin solution was drawn out with a pipette and tested bacteriologically. The solution proved to be still active.

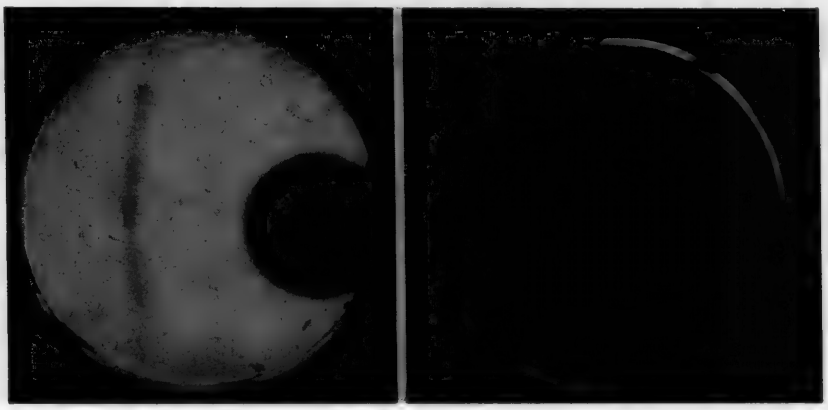
Eight roots from persons aged 44 to 54 years (mean age 49 years) were tested by this method.

Two roots from a woman aged 39 years were prepared and tested in an identical fashion, but an area about 1 mm. in diameter of the exposed root surface was burred in order to remove the cementum before penicillin was introduced into the root canal. These tubes were placed in agar with the areas from which cementum had been removed orientated to one side.

Results: Following incubation, in all cases there were zones of inhibition of bacterial growth in relation to the control windows but no areas of inhibition were observed in relationship to the group of 8 teeth (Fig. 2, *A* and *B*). On the other hand, well-defined circular areas of inhibition, centered over the areas from which cementum had been removed, were found in relation to the other 2 roots (Fig. 3). The eccentricity of these areas of inhibition in relation to the root was significant because it indicated that they were due to the diffusion of penicillin from the areas of dentin exposed by burring and not from the whole of the root surface.

Method 2: The first method permitted the study of only small zones of root surface. An apparatus was therefore designed to enable a much larger proportion of the root surface to be examined.

This apparatus (Fig. 4) was essentially a rigid clamp made of brass with two connecting posts (p) of duraluminium alloy. The design was such that roots (R) of various lengths could be secured under pressure between two rubber discs (d_1, d_2) which provided an effective seal. A fine-bore metal tube (t) pierced the rubber disc (d_2), providing a patent canal which connected the reservoir (r) with the root canal of the specimen under examination. The reservoir could be sealed off by means of the nut (s) and its rubber washer (w).



A. *B.*

Fig. 2.—Method 1. *A*, Petri dish after 24 hours' incubation showing result of test of root with intact cementum. Colonies of *Staph. pyogenes* have grown over the whole surface of the agar except for a wide zone around the control window. The root with attached tubes is obscured by the growth of organisms. *B*, Reverse side of the dish showing position of root and attached tubes.

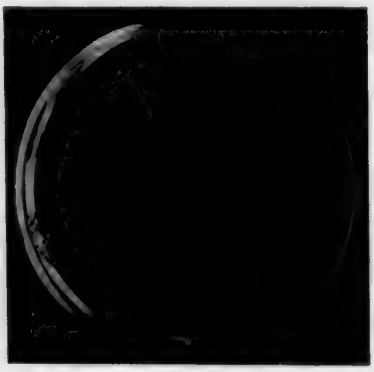


Fig. 3.—Method 1. Petri dish after 24 hours' incubation showing result of test of root from which a small area of cementum was removed. There is a wide zone of inhibition in relation to the control window. Eccentrically situated in relation to the zone of root between the rubber tubes is a circular area of inhibition centered over the small area (indicated by white dot and pointer) from which cementum was removed.

The crowns and apices of single-rooted teeth and some double-rooted premolars were removed with a fret saw and the canals enlarged with reamers. The coronal end of the root canal was slightly enlarged with a dental bur so

that it would fit closely over the metal tube (*t*). Each root was dried with sterile paper points and gauze swabs, or in a few cases by dipping in alcohol and passing through a flame. The root was then placed in the sterilized apparatus and secured under pressure. The root canal and reservoir were filled with penicillin solution (100 to 500 units per milliliter) by introducing a fine glass pipette through the opening provided by the removal of the sealing nut (*s*) and washer, which were then screwed tightly in position. This, in addition to making a leak-proof seal, produced a slight positive pressure inside the root canal and

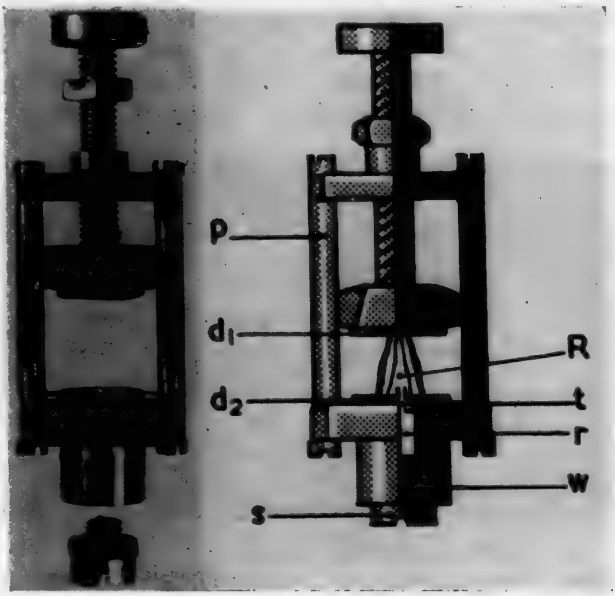


Fig. 4.—Method 2. *A*, Photograph of clamp. *B*, Sectional diagram of clamp. *R*, Root; d_1, d_2, rubber washers; *r*, reservoir; *t*, patent metal tube; *p*, duraluminum posts; *s*, sealing nut; *w*, rubber washer.

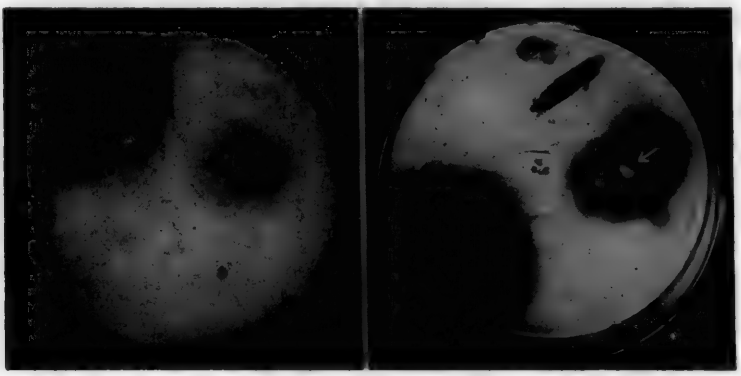
reservoir. The apparatus was placed into a deep Petri dish and just covered with nutrient agar. After being kept in a refrigerator for periods varying from 3 to 96 hours to permit diffusion of the penicillin, the agar surface was inoculated with *Staph. pyogenes*. A drop of the original penicillin solution was inserted in a control window in each case and the dish was then incubated at 37° C. overnight.

Four roots from persons aged 10 to 13 years dried with sterile swabs and 2 roots from persons aged 13 and 60 years dried in alcohol were tested by this method.

In addition 7 roots from persons aged 10 to 14 years dried with sterile swabs and 4 roots from persons aged 12 to 15 years dried with alcohol were prepared and tested in an identical manner, but an area about 1 mm. in diameter of the root surface was burred in order to remove the cementum. In these cases the

roots were secured in the apparatus so that their greatest diameter was horizontal when placed in the agar and the cementum was removed from one of the lateral surfaces.

Results: Following incubation there were zones of inhibition in relation to all the control windows. No areas of inhibition were observed in relation to any of the teeth with intact cementum (Fig. 5, *A*). In every case, however, where an area of cementum had been removed there was a well-defined area of inhibition eccentrically situated in relation to the root and centered exactly over the area of exposed dentin (Fig. 5, *B*). The close relationship between the areas of inhibition and the areas of exposed dentin was even more evident in these experiments than in Method 1, because in some cases two-rooted premolars of large diameter were used. There was thus no doubt that the areas of inhibition were due to the passage of penicillin through the exposed dentin.



A. *B.*

Fig. 5.—Method 2. *A*, Petri dish after 24 hours' incubation showing result of test of root with intact cementum. The position of the clamp is obscured by the growth of organisms over it and corresponds with the indistinct dark area to the right of the zone of inhibition around the control window. *B*, The result of test of root from which a small area of cementum was removed. There is a circular area of inhibition eccentrically situated in relation to the root and centered over the small area of exposed dentin indicated by pointer.

(B) *Permeability of Enamel.*—In some preliminary experiments the crowns were removed from teeth at the cervix. Most of the coronal dentin was removed with a dental bur under water to prevent drying and overheating. The crowns were then placed vertically in nutrient agar in Petri dishes with the cervices about 2 to 3 mm. above the level of the agar. After inoculation of the agar surface with *Staph. pyogenes,* penicillin solution containing 100 units per milliliter was carefully introduced into crown cavities with a fine pipette. Following incubation, areas of inhibition, often eccentrically situated, were observed in relation to the majority of the crowns. Some moistness of the cut dentin surfaces in relation to the zones of inhibition appeared to have developed during the period of incubation, and it was suspected that there had been some creeping of penicillin solution over the margins of the crowns. This series of experiments was therefore discarded.

A similar technique was employed in a study of a further 22 crowns from persons aged 35 to 59 years with a mean age of approximately 41 years. The cervical portions of the crowns were, however, pushed into sterile rubber tubes and secured with wire ligatures so that approximately one-half of the crown surfaces remained exposed. The crowns and attached tubes were embedded in agar to a level slightly above that of the wire ligatures (Fig. 6). It must be mentioned that in this series the crowns were dipped in absolute alcohol and momentarily flamed before the attachment of the sterile rubber tubes. Penicillin solution containing 100 units per milliliter was then placed in the tubes and crown cavities, following which the plates were kept in a refrigerator for periods varying between 12 and 48 hours to allow for diffusion. The agar surface was then inoculated with *Staphylococcus pyogenes* and a penicillin control window was cut in the agar. The plates were examined after incubation overnight.

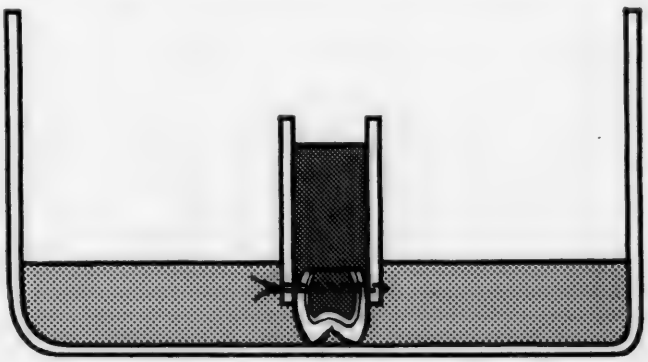


Fig. 6.—Diagram showing method of testing permeability of enamel, with tooth crown and attached rubber tube placed vertically in agar in a deep dish. The rubber tube is almost filled with penicillin.

In a further series consisting of 6 crowns from a person aged 61 years, 4 crowns from a female aged 30 years, and 1 premolar crown from a boy aged 13 years, treatment with alcohol was omitted.

Results: Zones of inhibition developed around all the control windows. No zones of inhibition were observed around any of the 33 crowns tested.

DISCUSSION

It would obviously be an advantage to study this problem in vivo, but it was not possible to devise such a method applicable to man. In most cases the interval between extraction of the teeth and the commencement of the experiment was less than 1 hour and when the interval was longer the teeth were stored in saline solution in a refrigerator. A proportion of the teeth used early in the investigation, before it was realized that sterility of the root surface was not essential, were dipped in alcohol and flamed. It may be argued that this treatment could have influenced the permeability of the tissues, but in 2 of the series identical results were obtained with a significant number of teeth not subjected to this treatment.

It was found that penicillin in a dilution of 50 to 100 units per milliliter was the optimum strength to provide a test of suitable delicacy. Where the clamp, which had to be buried slightly more deeply in agar, was employed, however, a stronger solution, up to 500 units per milliliter was sometimes used.

Our results show that under the conditions of our in vitro experiments penicillin inserted into pulp cavities does not penetrate the root and reach the root surface. We consider that it is reasonable to assume from these observations that the value of penicillin in root canal therapy for the treatment of periapical infection must be attributed to the passage of penicillin through the apical canals and possibly through the apical cementum which, at least in dogs and "monkeys," has been shown by Stewart-Ross[15] and Stones[16] to be permeable to dyes. Our experiments provide no information regarding the permeability of the root apex because the roots employed were devoid of apices.

Our results appear to be in conformity with the observations of Stewart-Ross and Stones, which suggest that there is an impermeable barrier in the region of the cementum-dentin junction of the greater part of the root. The divergence between our results and those of Shuttleworth[13] could be due to his reliance on wax as a sealing agent.

Some recent work with radioactive isotopes shows that the cementum of man and cats is in vivo permeable to radioactive iodine (I^{131}) in the form of sodium iodide (Bartelstone[2, 3] and Wainwright[19, 20]). Wainwright,[18] using extracted human teeth, has found that the outer cementum surface takes up a variety of radioactive substances. It is, however, at least doubtful whether any of these molecules pass from the cementum into dentin and vice versa. In this connection we feel compelled to mention that the work of Götte, Hattemer, and Frimmer,[8] who sealed radioactive arsenic (As^{76}) in the form of arsenic trioxide in contact with the coronal pulp and showed by radioautography that the arsenic entered the dentin of the crown, has been erroneously adduced by Wainwright[19, 21] as evidence that arsenic can pass from the dentin, through cementum and into the periodontal membrane. Götte, Hattemer, and Frimmer merely speculated on the ultimate result of arsenic remaining indefinitely in the dentin of teeth devitalized with arsenic and possibly eventually diffusing out into the periodontal membrane and exerting a toxic effect.

Although the permeability of structures to molecules is dependent upon the relationship between the pore size of the structure and the sizes of the molecules concerned, which in turn are approximately proportional to their molecular weights, many other factors are concerned, such as molecular shape and the specific chemical groupings possessed by the molecules. Nevertheless, since the molecules of streptomycin (streptomycin sulfate, m.w.1457.4; streptomycin hydrochloride, m.w.691), Aureomycin (m.w. approximately 478), Terramycin (m.w. approximately 460), and chloramphenicol (m.w.323.1) are of the same order of magnitude as penicillin (m.w.356.4), it seems probable that our conclusions could be extended to include these antibiotics.

It is not our intention to enter deeply into the long-standing controversy concerning the permeability or otherwise of enamel, but merely to report and evaluate our findings which were a by-product of our study of root permeability.

Berggren[5] has thoroughly reviewed the earlier literature and studied the in vivo and in vitro permeability of enamel to aniline dyes, tetanus toxin, and radioactive isotopes in man and other animals. He found that dyes penetrate enamel in a centrifugal direction erratically and incompletely. Tetanus toxin would pass centripetally through the enamel of young dogs in vivo provided it was applied to the crown surface in association with glucose (see also Berggren and Hedström[6]). On the other hand, Sognnaes and Shaw[14] found that in rhesus monkeys the centripetal passage of radioactive phosphorus(P^{32}) in saliva was reduced by the addition of 20 per cent sucrose. Berggren[5] also found that enamel was permeable in both directions to radioactive sodium (Na^{24}) in the form of sodium chloride and radioactive phosphorus (P^{32}) in the form of acid sodium phosphate. Bartelstone,[2, 3] from in vivo experiments on cats and man using radioactive iodine (I^{131}) in the form of sodium iodide, concluded that enamel is permeable to iodine. Sognnaes and Shaw,[14] in experiments on rhesus monkeys, obtained similar results and, by assessing the radioactivity of various fractions of enamel and dentin, showed that when radioactive phosphorus (P^{32}) is injected into the blood stream there is a decreasing gradient in the dentin from within outward and a decreasing gradient in the enamel from without inward. The gradient in dentin was almost completely abolished by extirpation of the pulp and the gradient in enamel was abolished by isolation of the teeth from saliva. They concluded that the passage of radioactive phosphorus from the saliva into enamel was mainly responsible for the radioactivity of the enamel. Wainwright and Lemoine[17] showed by in vitro experiments on human teeth that enamel is penetrated by carbon-labeled urea (C^{14}), or at least by the carbon isotope. On the other hand, Wainwright,[18] using radioactive isotopes, found that the enamel of freshly extracted human teeth was not permeable from the outer surface to a variety of inorganic salts. Atkinson,[1] on the basis of osmotic experiments in vitro on human teeth, concluded that enamel behaves as a semipermeable membrane.

Jansen and Visser,[10] who demonstrated the permeability of the enamel of living dogs to a fluorescent dye, have drawn attention to certain confusions that exist in the literature and in particular to the need for caution in deducing in vivo behavior from in vitro experiments on permeability.

It is apparent that the laws governing enamel permeability have yet to be established. Our results show that under the conditions of our experiments penicillin does not pass through the whole thickness of human enamel, but they do not show to what extent various parts of the thickness of enamel may be permeable to penicillin solutions.

SUMMARY

The literature relating to permeability of the dental tissues is briefly reviewed. Penicillin solution was placed in the pulp cavities of the roots of freshly extracted human teeth from persons of a variety of ages. In 8 cases the pulp cavities were sealed by means of wired-on rubber tubes and in 6 cases a metal clamp with rubber washers was employed as a sealing device. No penicillin was detected bacteriologically on the root surfaces after periods of up to 96 hours,

but in 13 cases where areas of cementum were removed with a dental bur penicillin penetrated to the surface of the exposed dentin. Penicillin was inserted in the enlarged pulp cavities of the caries-free crowns of 33 teeth. No penicillin was detected on the outer enamel surfaces after periods up to 48 hours.

The deduction is made that when penicillin or other antibiotics are inserted in root canals therapeutically the antibiotic only penetrates to the periodontal membrane in the region of the apical canals. Under the in vitro conditions of these experiments penicillin does not pass centrifugally through the whole thickness of human enamel, but to what extent various parts of the thickness of enamel are permeable to penicillin solutions has not been determined.

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THE SOURCE OF MINERAL SALTS IN SUBGINGIVAL CALCULUS

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WHILE it is generally agreed that the organic matrix of calculus (both supragingival and subgingival) is built up by microorganisms—mainly actinomyces and leptothrix[10]—there are 2 opposing opinions on the source of the mineral salts in subgingival calculus. One group[2, 6, 8, 9, 11, 12, 14] contends that they are precipitated from the saliva, and that the saliva is constantly circulating to the bottom of even very deep pockets. Another group[1, 3-5, 7, 10, 13, 15] maintains that the mineral salts in subgingival calculus are derived from the blood, being precipitated from exudate and pus entering the pocket from the surrounding soft tissue. No experiment proving that saliva can or cannot enter into gingival pockets has, however, been undertaken.

For the further study of the formation of subgingival calculus, it is of the greatest importance to make sure from which of the previously mentioned sources the mineral salts are derived. As there are no means by which it can be decided whether or not saliva penetrates into normal and pathologic pockets, an investigation was made with the aim of finding out to which extent vital dyes are able to do so. A study of this type should contribute to throwing some light upon the circulation in the pockets.

MATERIAL AND METHOD

Eighteen teeth in 11 individuals between 42 and 69 years of age represented the human material. The teeth, which had to be extracted for a replacement by full dentures, were all surrounded by deep pockets. Calculus covered the teeth from 1 to 8 mm. below the gingival margin (Fig. 1). Copper rings which fitted close to the gingiva were cemented onto these teeth. Before the fastening, dry cotton was pressed between the copper rings and the crowns, allowing only enough space for the cement. The cement having set, the cotton inside the tube was saturated with different dyes by means of a syringe, the thin needle being inserted between the copper band and the gum. The tubes were allowed to remain in place for 6 to 10 hours, when they were removed very carefully. In all cases it was verified that there was still plenty of dye left in the cotton, the patients having been instructed not to suck on the tube to drain it. The surplus of dye was then removed from the gingiva by a gentle stream of lukewarm water. Every precaution was taken, though, not to rinse inside the pockets. By means of a heated stream of air, the gingival tissue was dried carefully. The gingiva was then examined in strong light through a magnifying glass. Eventually the teeth, which were all comparatively loose because of periodontal involvement, were extracted, rinsed in water, and dried by means of hot air. The teeth were then immediately examined under the stereomicroscope.

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For the investigation, 2 young dogs with healthy gingival tissue were used also. There was no formation of subgingival calculus, and the pockets which measured 1.5 to 2.5 mm. ended at the cementoenamel junction (Fig. 2.) Impressions of the jaws were taken in modeling compound. These impressions were replaced after having been moistened with the dye used. The dye was renewed every hour. The observation period was in both cases 24 hours, and the animals

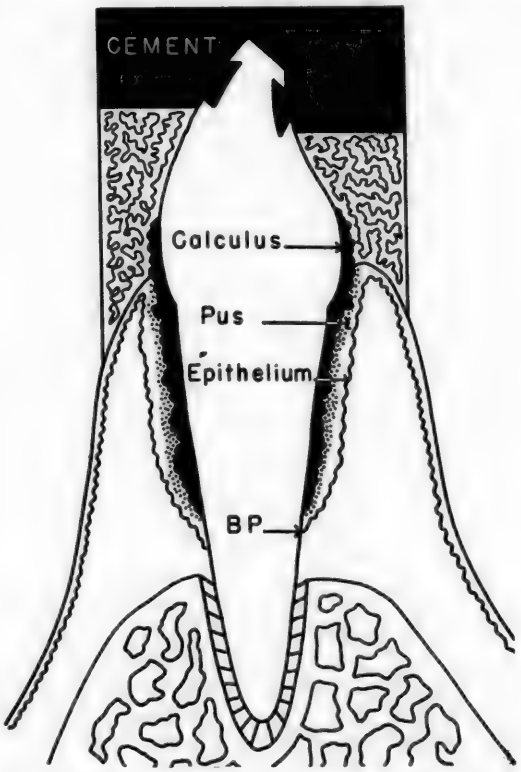

Fig. 1.—Schematic drawing illustrating tooth surrounded by deep pocket. Tooth covered by subgingival calculus reaching near to bottom of pocket, *BP*. Space between epithelium and calculus filled with pus. No open space. Copper ring fitting tightly to gingiva fastened to tooth by means of cement retained in undercuts. Space between cement and gingiva filled with cotton saturated with vital dye after hardening of cement.

were kept anesthetized all the time. The animals were then sacrificed and the jaws were carefully taken out. The jaws were allowed to dry on the surface, whereupon an examination in the stereomicroscope was undertaken. Finally a dissection was performed. Methylene blue, toluidine blue, and alizarin red were each used in 6 cases in the human material. Methylene blue and alizarin red were used in the dog material. All the dye solutions were saturated.

OBSERVATIONS

The whole mouth was deeply stained, not only the area covered by the ring. When the gingiva was pressed aside to allow a view into the pocket it was ob-

served, however, that not the slightest trace of the dye could be found below the gingival margin either on the tooth or on the soft tissue. This observation was consistent both in the human material and in the dogs. The distinct border between the stained part of the tooth above the gingival margin and the unstained part below it could be even more clearly demonstrated when the teeth were examined under the stereomicroscope.

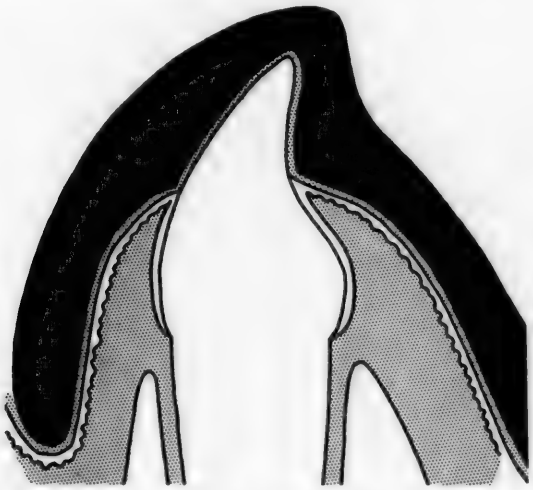

Fig. 2.—Schematic drawing illustrating tooth surrounded by healthy pocket, ending at cementoenamel junction. Epithelial cuff (epithelial attachment) lying in close contact with enamel surface to gingival margin. No open space. Impression (black) taken of jaws and teeth. Impression replaced after having been moistened with vital dye.

DISCUSSION

The consistent finding in this experimental series was that none of the 3 dyes could be traced in the pockets. This should mean that molecules of the size occurring in methylene blue, toluidine blue, and alizarin red do not usually penetrate into pockets by diffusion, as there should hardly be any reason to believe that all these dyes were decomposed as soon as they had penetrated below the gingival margin. There is a possibility that smaller molecules might have penetrated more easily, but it is hardly probable. Neither is it likely that an elongation of the observation period would have made any definite difference. On the basis of these observations it must, therefore, be questioned whether even the saliva ever penetrates below the gingival margin without being brought there by mechanical means. The explanation seems to be that from all pockets there is a constant flow of cellular elements and tissue fluid.[17] In healthy pockets where a normal epithelial cuff[16] is closely fitted around the tooth to the gingival margin, this secretion is rather minute. But even if very slow, this stream most likely proceeds outward much faster than the saliva penetrates inward by diffusion. This conclusion is corroborated by the fact that India ink inserted into healthy pockets is completely removed from these in the course of about 48 hours.[16] It should here be especially emphasized that a normal pocket is but a potential

pocket that has to be opened by mechanical means. Healthy epithelial cells always border directly upon the tooth surface even if they are not attached[16] to it, as is often maintained (Fig. 4). There is, accordingly, no open space in which a diffusion can easily take place.

The outward stream from the pocket is manifoldly increased when an irritant causes an exudation into the pocket, and subgingival calculus with its bacterial plaque is definitely such an irritant[17] (Fig. 3). It is a well-known clinical observation that pus is constantly drained out of deep pockets with

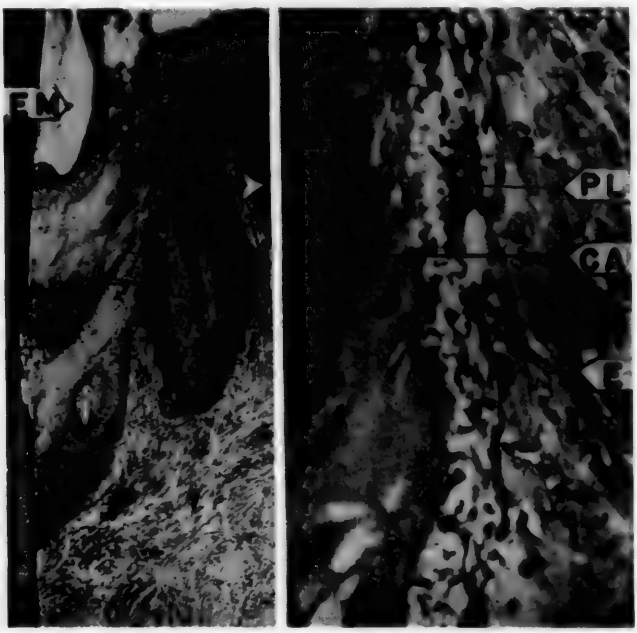

Fig. 3.—Pathologic pocket with subgingival calculus in dog. Between epithelium (*E*) and bacterial plaque on surface of calculus (*CA*) are polymorphonuclear leukocytes (*PL*) on their way out of the pocket. No open space. *EM*, enamel space.

subgingival calculus, and it is easy to understand that this stream runs much faster than the molecules from the saliva are able to pass into the pocket by diffusion. Thus it is most likely that the subgingival calculus is bathed in fluid exclusively originating from the blood. And as blood contains ample supplies of mineral salts, there should be no difficulty whatsoever in explaining the presence of these salts in subgingival calculus. This is also corroborated by the fact that calcification of dead and dying tissue occurs in many other places in the body. No conclusions concerning the mineralization of *subgingival calculus* can, therefore, be drawn from observations made on saliva alone, until it is definitely proved that saliva really penetrates into pockets. In future biochemical studies of the mode of calcification of subgingival calculus, attention should, therefore, also be paid to the blood as a possible source of the mineral salts.

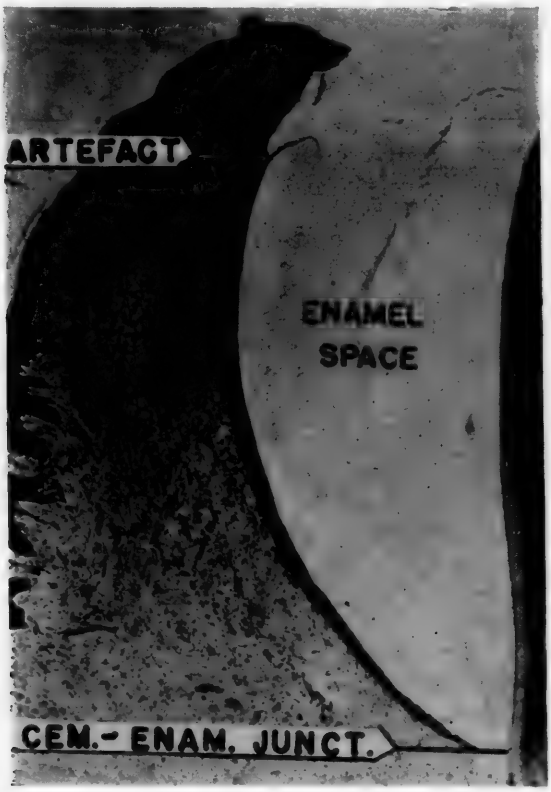

Fig. 4.—Healthy gingival pocket in dog. Normal epithelial cuff closely adapted but not attached to enamel (disappeared during decalcification). Bottom of clinical pocket at cementoenamel junction. Split in upper third of epithelium is artefact, and clinical pocket is not limited to this area.

SUMMARY AND CONCLUSION

An experimental investigation is reported in which attempts were made to have different vital dyes penetrate into healthy and pathologic pockets. The tooth and the gum margin were bathed in dyes for 6 to 10 hours in man and for 24 hours in dogs. The consistent observation was that the dyes in no case penetrated below the gingival margin. The conclusion is drawn that, most likely, the saliva will not penetrate below the gingival margin, and that the source of the mineral salts in subgingival calculus is the blood.

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PERIODONTAL FIBER REATTACHMENT IN REPLANTED INCISORS OF THE MONKEY

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THE enamel epithelium has been found to readhere to the enamel after detachment in monkeys[1] and epithelial cells have been shown to attach to gold, porcelain, and acrylic crowns.[2] Periodontal fibers have been found to reattach by the formation of new layers of cementum which engulf the free ends of the periodontal fibers.[3-5]

Ramfjord[6] has shown in monkeys that both connective tissue and epithelial reattachment occurred on root surfaces which had been first covered by bands and later exposed in chronically inflamed periodontal pockets. "No reattachment occurred from the time the bands were removed until the epithelial lining of the pocket was cauterized and scraped away." This article does not show to what extent reattachment will occur under very adverse conditions, as in instances where the organic material and superficial dentin might be burred away or where the tooth's root might be exposed to chemicals.

There, thus, seemed justification for studying extracted teeth which were drastically treated prior to replantation. Such a study might very well provide information on whether or not the epithelium destroys the connective tissue in a pocket or extends along the surface only after the connective tissue fibers are detached, because of infection or other causes.

Equally important is whether the failure of reattachment is because of changes in the cementum and its surface or because of changes in the periodontal membrane.

METHODS

The permanent incisors of monkeys (Rhesus) were used in these experiments. The teeth were extracted and in most instances, the pulps were removed via the apex. The teeth were then subjected to one of the following treatments: burring off the organic material from the labial or lingual side; burring notches into the dentin; or immersing in either 10 per cent formalin, 95 per cent alcohol, or hot water (60° C.). The teeth were then replaced in their original alveoli and left without splinting.

After a lapse of time varying from several days to months, the monkeys were sacrificed and the jaws usually fixed in Bouin's fluid. Following decalcification and paraffin imbedding, sections were cut and stained by various methods.

OBSERVATIONS

Conditions After Extracting and Immediately Replanting.—In these experiments, teeth were extracted; the pulps were removed from some via the

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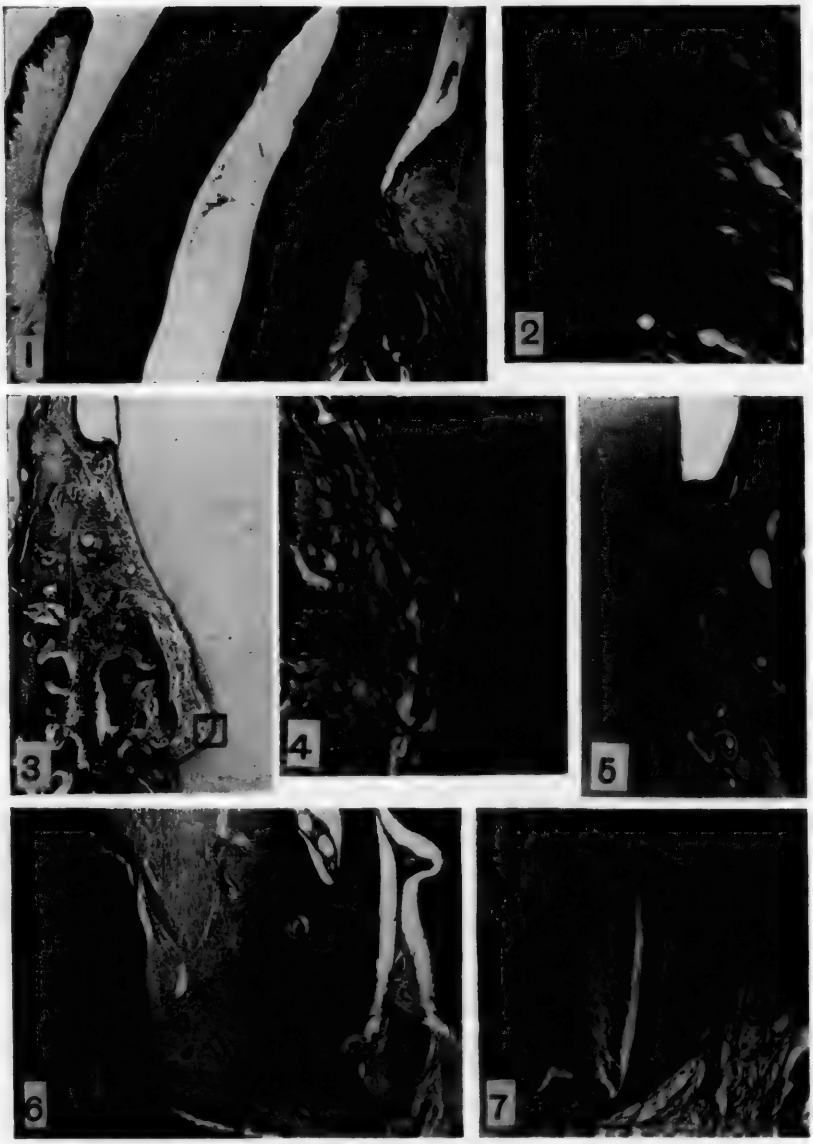

Fig. 1.—Extracted, pulp removed, replanted. Sixty days later, tooth prepared. Abscess at base. No regeneration of pulp. *1*, Lymphocytic infiltration (×15; reduced ¼).

Fig. 2.—Enlargement of area marked in Fig. 1 showing reattachment in the cementum (×470; reduced ¼).

Fig. 3.—Cut was made in dentin with dental burr. Thirty days later, tooth was prepared (×40; reduced ¼).

Fig. 4.—Enlargement of area marked in Fig. 3, showing reattachment of periodontal fibers into cementum (×450; reduced ¼).

Fig. 5.—Extracted ,pulp removed, burred off with Chayes stone. Thirty-three days later, tooth prepared. Nonodontoblastic pulp had regenerated and periodontal fibers had reattached; ankylosis and resorption had occurred (×35; reduced ¼).

(*Legends continued on following page.*)

apical approach, while the pulps in others were not altered before replantation. Fig. 1, a typical condition, shows a tooth which was returned to its alveolus for 60 days after extraction and pulp removal. A large apical abscess had prevented pulp regeneration. Associated with this apical abscess was considerable resorption. Despite the infected apical area, the periodontal membrane had reattached throughout its entire length. Additional cementum had been deposited in which the periodontal fibers appear to have normal attachment (Fig. 2). The periodontal fibers have reattached beyond the coronal limit of the alveolar bone. On the lingual side may be seen an infiltration of lymphocytes beneath the epithelium. Associated with this lymphocytic infiltration are epithelial outgrowths into the underlying tissue. There was no ankylosis.

Teeth replanted for 120 days showed, with the exception of occasional resorption areas along the root, few differences from those left for shorter intervals. Where the pulps have been left intact, they had persisted in many cases. Where they had been removed and there were no apical abscesses, there was much regeneration of nonodontoblastic pulp into the pulp canal. Periodontal fibers had reattached along the entire root and coronally beyond the extent of the alveolar bone. Associated with subepithelial lymphocytic infiltration (Fig. 12) were many rete pegs. In this specimen, there has been an extensive growth of the crevicular epithelium downward along the root to a small area of resorption.

Conditions Following Extraction, Burring Away Organic Material and Superficial Dentin Before Replanting.—Fig. 3 shows a tooth which was not extracted. The gingiva was retracted, and a cut was made into the dentin. No infection followed, and there has been good fiber attachment. In the deepest part of the cut, considerable cementum has been deposited (Fig. 4).

When the teeth were extracted, the organic material burred away with a Chayes stone, and the teeth returned to their alveoli, one found areas of normal attachment, ankylosis, and resorption throughout the extent of the periodontal membrane. Fig. 5 illustrates such changes, after an interval of 33 days. The pulp, devoid of odontoblasts, had regenerated into the pulp canal. The relations of the gingival epithelium were quite normal. Areas of resorption were interspersed along the root of the tooth with intermittent regions of ankylosis.

Where more drastic alterations were performed, such as burring away superficial portions of the dentin, or burring grooves in the extracted teeth before replanting, cementum was deposited on the altered surfaces, and the periodontal fibers were firmly anchored. Fig. 6 shows such a condition midway of the length of the root 60 days after the operation while Fig. 7 shows cemental deposition at the apex 22 days after returning a tooth to the alveolus. In the

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Fig. 6.—Extracted, pulp removed, groove was made in dentin midway of root of tooth, replanted. Sixty days later, tooth was prepared. Note cemental deposition. *c*, Cementum (×50; reduced ¼).

Fig. 7.—Extracted, dentin burred away at apex of tooth, replanted. Twenty-two days later, tooth prepared. Pulp has survived, and cementum has been deposited by periodontal membrane. *c*, Cementum (×100; reduced ¼).

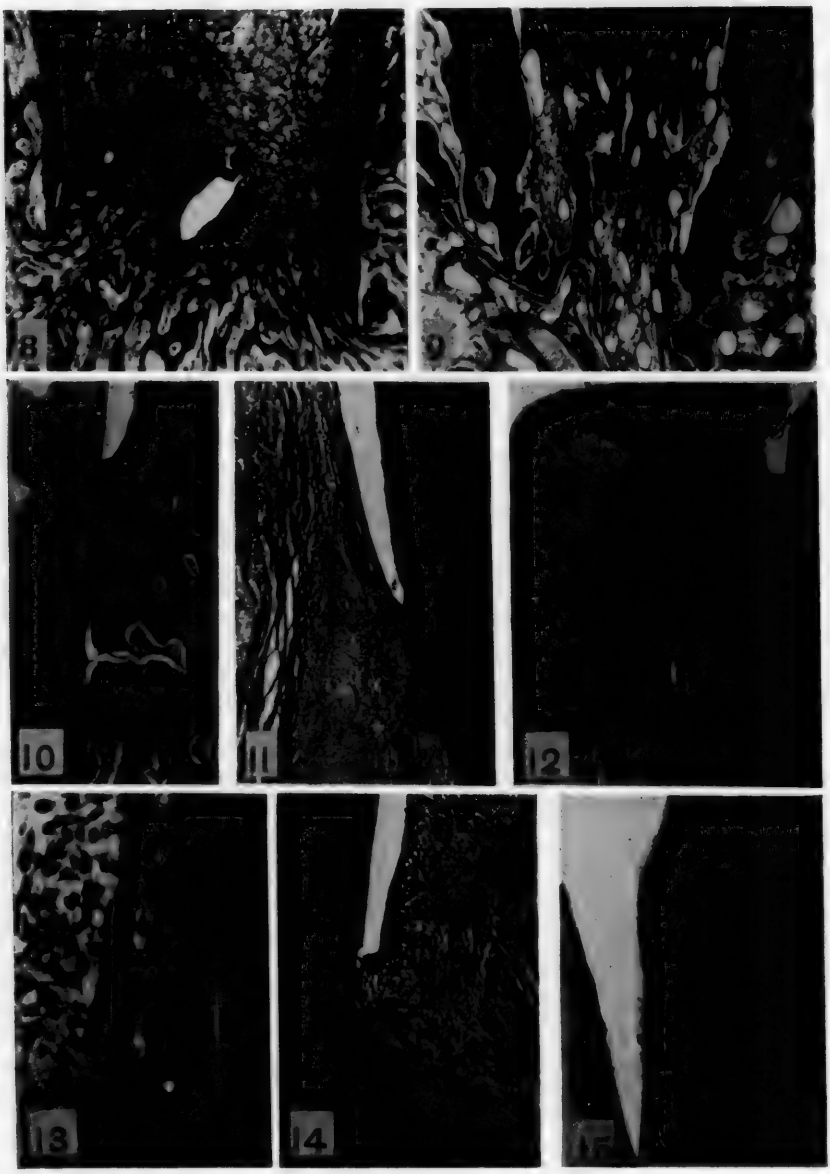

Fig. 8.—Extracted, pulp removed, immersed in 10 per cent formalin 10 minutes, washed in H_2O 15 minutes, replanted. Fifteen days later, tooth prepared. Periodontal fiber attachment and ankylosis present (×30; reduced ¼).

Fig. 9.—Extracted, immersed in 10 per cent formalin for 20 minutes, washed in H_2O 15 minutes, replanted. Sixty-four days later, tooth prepared. Much resorption has occurred at apex (×22; reduced ¼).

Fig. 10.—Extracted, pulp removed, immersed in 95 per cent alcohol for 10 minutes, then in water for 10 minutes prior to replanting. Forty-three days later, tooth prepared. Extensive ankylosis is present on lingual side of tooth (×28; reduced ¼).

(*Legends continued on following page.*)

last instance, the pulp was not removed prior to the replanting, and the odontoblasts have survived.

Conditions After Extraction and Formalin Treatment Prior to Replanting.—After extraction and pulp removal, the tooth in Fig. 8 was placed in 10 per cent formalin for 10 minutes, and washed for 15 minutes in distilled water prior to replanting. Fifteen days after replanting, it was removed, fixed, and sectioned. The periodontal fibers have reattached, and ankylosis areas may be found intermittently in the periodontal membrane. No resorption is evident. Nonodontoblastic pulp has filled approximately one-third of the pulp canal. There was no lymphocytic infiltration in the subepithelial tissue around the gingival crevice.

After treatment in formalin for 20 minutes and a longer interval of 64 days of replantation, much more resorption is found (Fig. 9). Much of the apex is resorbed, yet there is no pressure on the tooth from the surrounding bone. Nonodontoblastic pulp has filled the entire pulp cavity. Areas of ankylosis are common along the root, and relations of the gingival epithelium are normal. Such teeth show conditions typical of the many teeth treated with formalin.

Conditions Following Extraction and Immersion in Alcohol or Hot Water Prior to Replanting.—Fig. 10 shows a tooth which was immersed in 95 per cent alcohol for 10 minutes, then washed in water for 10 minutes prior to replanting. Forty-three days later, upon removal and sectioning, a normal periodontal membrane was found on the labial side, while there is extensive ankylosis on the lingual side. Living pulp, containing a few bone spicules, filled the pulp cavity. There was little resorption of the tooth's root, and alcohol immersion does not seem quite as drastic a treatment as formalin immersion. Much infection followed immersion in hot water prior to replantation. Despite the fact that the entire apex was badly infected, the periodontal membrane remained firmly attached coronally in most cases (Fig. 11). Gingival epithelial relations were re-established and appear normal.

Conditions Affecting the Growth of the Crevicular Epithelium.—Much epithelial growth from the crevicular epithelium into the underlying connective tissue has been encountered. This growth was in the form of pegs and, in most instances, it was associated with lymphocytic infiltration (Figs. 1, 12, 13, 14, 15). In Fig. 12, a peg has extended down along the root surface to a small area of resorption. An enlargement of the latter relations is presented in Fig. 13, which shows many lymphocytes, although toward the region of resorption only

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Fig. 11.—Extracted, immersed in hot water for 10 minutes, replanted. Sixty days later, tooth was prepared. Much infection and resorption is present around the apex. Periodontal relations are normal near gingiva (×50; reduced ¼).

Fig. 12.—Extracted and immediately replanted. One hundred twenty days later, tooth prepared. Shows enamel epithelial pegs associated with lymphocytic infiltration (×32; reduced ¼).

Fig. 13.—Enlargement of section marked out in Fig. 12, showing epithelial downgrowth along the root (×450; reduced ¼).

Fig. 14.—Extracted, immersed in hot water for 10 minutes, replanted. Sixty days later, tooth prepared. Epithelial downgrowth exists where the connective tissue fibers are not attached (×50; reduced ¼).

Fig. 15.—Extracted, pulp removed, replanted. Sixty days later, tooth prepared. Note that pegs are confined to area where lymphocytic infiltration exists (×50; reduced ¼).

epithelial cells are found. In Fig. 14, the epithelial downgrowth extends to the point where the periodontal fibers are well attached. In Fig. 15, the growth of the pegs is confined to the area where the lymphocytic infiltration exists.

DISCUSSION

Despite apical infections in many of these experiments, periodontal fiber relations along the root were quite normal. Periodontal reattachment existed coronal to the alveolar bone, so the support of the bone is not absolutely necessary for the fiber attachment to the cementum.

Immersion in formalin, alcohol, or hot water certainly altered the superficial portion of the root of the tooth, but the tissue cells of the periodontal membrane were potentially able to supply cementum in which the periodontal fibers were engulfed. Vitality, thus, is not a decisive factor for reattachment, as pointed out by Ramfjord.[7] Clean, sterile cementum would seem to be important, and if such conditions are established in a periodontal pocket, reattachment of the fibers is possible.

Areas of ankylosis were found adjacent to regions of normal-appearing periodontal fibers and often next to areas of resorption. The areas of ankylosis were larger and more common on the lingual side of the tooth. Since the teeth were usually tipped in this direction, creating pressure, ankylosis was not expected.

Equally unexplainable is the presence of areas of resorption, which were interspersed among regions of normal periodontal fibers. Pressure does not seem to be directly responsible for the resorption since the apexes of teeth have been resorbed when surrounded by very cancellous bone, and resorption areas are found coronally to the alveolar crest. Osteoclasts were seldom found in the vicinity of the areas of resorption. Resorption took place through the cementum, so the latter cannot be regarded as protective. Ankylosis was more frequently encountered in teeth immersed in formalin and alcohol. Resorption was greatest in teeth treated with formalin.

Epithelial pegs from the crevicular epithelium were usually found associated with lymphocytic infiltration. When these conditions existed, epithelial downgrowths along the root and the lack of connective tissue attachment to the cementum were found. Investigators have interpreted these conditions differently. Goldman[8] concluded that the prevention of apical proliferation of epithelium is dependent upon the maintenance of the insertion of the principal fibers of the periodontal membrane, and that epithelial proliferation can take place only through the destruction of these connective tissue fibers. Ramfjord[6] pointed out that an infected environment is not conducive to cementoblastic activity and the attachment of collagenous fibers, and that where there is infection, there will be no connective tissue attachment, but epithelial downgrowths will develop.

As exhibited in Figs. 12 and 14, it can neither be stated that the epithelial downgrowths along the root are responsible for destruction of connective tissue fibers nor can it be stated that the epithelium extends along the surface only

after the connective tissue fibers are detached because of infection or other causes, since both processes are occurring at the same time. The epithelial pegs and lymphocytic infiltration are commonly found together, and one may conclude that inflammation is present. Common to all these conditions, that is, an inflammation, epithelial invasion, and lymphocytic infiltration, is a low pH. Epithelial cells grow best at a pH of 6.9.[9] While this level of pH is conducive to epithelial growth, it is not favorable for the best connective tissue activity. Parshley and Simms[9] have found that a reduction of calcium and an increase of phosphate concentration, as compared with concentrations found to be optimum for adult fibroblasts, greatly increase epithelial growth. The pH of saliva varies between 6.4 and 7.4,[10] and the environment in the gingival crevice may very well be conducive to epithelial growth and destructive to connective tissue activity.

If pH may be a factor in the development of the periodontal pocket, one immediately queries if it can be responsible for ankylosis, resorption, and a normal periodontal membrane along the same side of the root as is shown in Fig. 5. Paff[11] presented evidence that bones grown at a pH of 7 to 7.3 contain distinctly more calcium than bones grown at pH 7.8 to 8. It hardly seems possible that a difference of pH could exist in such a short interval along the root of the tooth which would be conducive to the different structures. Then, too, it does not seem at all possible that the pH would be of the same level over a sufficient period to cause ankylosis. In bone development, adjoining areas of resorption and bone formation are common, and the pH could scarcely be of sufficient difference to account for these different processes. Pressure from tipping the tooth is generally accepted as one cause of resorption. In many instances in the present experiments, the tooth has been tipped toward the bone and the exact opposite (ankylosis), or bone formation, has resulted. Changes in the surface of the tooth which are not understood must be conducive to resorption and ankylosis. Formalin treatment in the present experiments appeared to be able to cause these changes.

In the reattachment of the periodontal membrane, fibers loosened from the cementum cannot be reattached directly to this calcified structure. Fibers were reattached in a new layer of cementum. This process does not necessitate the formation of new fibers but does involve the change of collagenous fibers into homogenous collagenous material in which calcium salts are deposited. In the present experiments, there has been little resorption of the alveolar bone.

CONCLUSIONS

1. Reattachment of the periodontal fibers to the tooth has been found to occur in replanted teeth from which the organic material and superficial dentin were burred away, or in teeth which were immersed in 10 per cent formalin, 95 per cent alcohol, or hot water (60° C.) prior to replanting.

2. The reattachment occurred by the formation of new layers of cementum in which the free ends of the periodontal fibers are engulfed.

3. Areas of ankylosis and resorption were found along the root surface of the replanted tooth. Inflammatory areas were mainly confined to the apex and gingival sulcus.

4. Inflammation was less common, while ankylosis was more frequently encountered, in the teeth treated with formalin and alcohol. Resorption was greatest in teeth treated with formalin. There is no evidence that resorption is directly the result of pressure.

5. It is suggested that the pH of the gingival crevice may be conducive to epithelial growth and destructive to connective tissue, resulting in the development of the periodontal pocket.

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THE SCREENING OF MATERIALS FOR ADHESION TO HUMAN TOOTH STRUCTURE*

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MANY reports have described clinical difficulties with the presently available resin filling materials.[1-4] It is possible that a partial solution to these problems can be found in the development of a plastic that would bond or adhere permanently to human tooth structure. For example, if the material adhered to the cavity walls during polymerization, the shrinkage might be controlled so that it would occur only in the body and on the surfaces of the filling which do not contact tooth structure, thus preventing separation at the margins. Good bonding might also prevent the "percolation" phenomenon, as described by Nelsen, Wolcott, and Paffenbarger,[1] and might serve to prevent the lodgment of oral microorganisms between the filling material and tooth structure after completion of the restoration. The desirable property of adherence to tooth structure has been sought, and tested for, among a large number of monomers, polymers, copolymers, and fillers, either singly or in combinations.

TENSILE TESTING

The large number of potentially adhesive materials, in the field of plastics, and the difficulty in preparing sections of tooth structure of a standard size necessitated the use of a strictly qualitative test for the preliminary survey. Quantitative testing was planned for materials that gave satisfactory results in the screening tests. The test used in this study was patterned directly after Paffenbarger, Sweeney, and Isaacs.[5]

Erratic results were obtained with ¼ inch diameter cylinders of ivory, in part due to swelling of the ivory in water. Preparations were made from human teeth that had been extracted not more than 30 days previously. They were selected and ground to fit in the hollow end of each of the sliding braces of the tester (Fig. 1). After they were fitted into the hollow end, a small hole was drilled through the brass tester and the tooth itself, and a steel pin inserted to hold the tooth in place during the test. Quick-set denture material was inserted to fill the space around tooth and pin (Fig. 2).

The contacting surfaces of the tooth specimen were paralleled in a machine jig using a 2/0 grit carborundum for the abrasive surface. The contacting surfaces were ground to approximately ¼ inch diameter in the preparation of all tooth specimens. Specimens were kept moist by repeatedly dipping in water; when not actually in use, they were stored in water.

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Procedure for the screening test was as follows: (1) The tooth surfaces were cleaned with cleansing tissue, wiped with 95 per cent alcohol, and allowed to dry. (2) Both tooth surfaces were moistened with a drop of the monomer or liquid being used. (3) The powder and liquid were mixed according to manufacturer's directions, or in the case of experimental mixes, for 30 seconds and in a thin consistency, as would be used for clinical cementation of restorations. (4) The slurry was placed between the 2 surfaces to be adhered. (5) A 10-pound weight was placed on top of the tester to hold the specimens together and the excess of cement was removed from the joint. (6) After 15 minutes, the tester was placed in water for 5 minutes. (7) The tester was now placed in a simple tensile device and the force in pounds required to separate the 2 halves was recorded (Fig. 3). Note that torsion is prevented by the accurately machined sleeve in which the 2 members of the tester slide. (8) All foreign material was removed mechanically from the surface of the specimens and each surface reground on the mechanical jig preparatory to the next test.

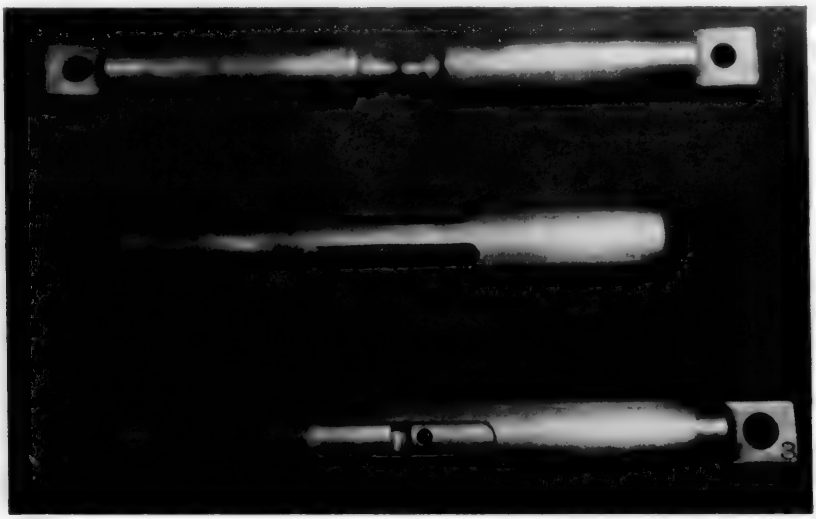

Fig. 1.—Tooth sections are mounted in assembled device. Disassembled tester contains gold test specimens.

Early in the work, it was discovered that the adhesion of some materials to enamel was much greater than to dentin; thereafter tooth specimens were prepared in such a fashion that their surfaces presented either only enamel or only dentin to the opposing specimen. During the course of the investigation more than 5,500 single tests were made. The results obtained with a representative group of materials are presented in Tables I, II, III, IV, and V. The materials tested were Acrylite–Fluoro Adhesive, Acrynamel, Dura Cement, Dura Kore, Grip Cement, Kadon, Kadon-Colorfast, Kayon, Just; Resin Cement, Pearlon, P.F., Sevriton, Smith's Resin Cement, Smith's Zinc Cement,

Texton, S.S. White Zinc Cement. Two or three hundred of the first tests on tooth specimens (which were carried directly to the testing machine after the cementing layer had set) indicated that dry testing has no relation to clinical conditions. Some materials which gave zero readings after 5 minutes' immersion in water required as much as 75 pounds of tensile pressure to separate the specimens when tested dry.

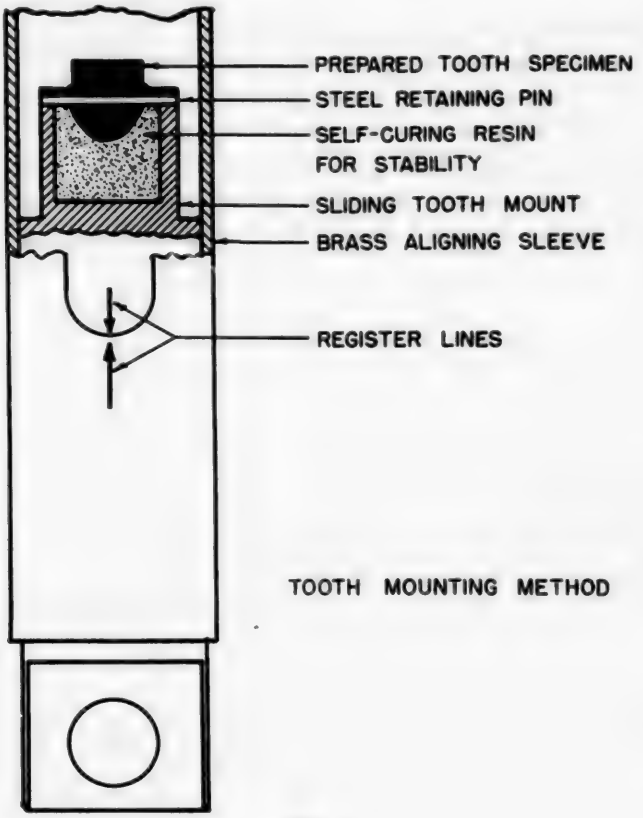

Fig. 2.

In general, it was found that finely divided fillers such as aluminum oxide and calcium carbonate had some tendency to improve the adhesion of polymers, probably because of the improved wetting and improved adaptation of the material to the surface of the teeth. Although no work has been done directly on evaluating the wetting of tooth surfaces, this should prove a fruitful study in the pursuit of adhesive materials; almost invariably, materials which wet the surface easily and quickly showed somewhat better adhesive properties. Preliminary dilatometric studies in the laboratory indicate that some fillers can be used to decrease the thermal coefficient of expansion of resins.

In general, it was found that very few of the products available to the profession today consistently maintain adhesion after immersion in water for 5 minutes (Tables I, II, and III). After 16 hours of immersion in water, readings on the tester were invariably zero with present materials. These results are contrary to those of Bartram,[6] who showed some adhesion to dentin under "moist" conditions for resin filling materials.

Fig. 3.—Tensile device.

As this paper was being finally prepared for publication, 2 new resin cements were made available to the profession. On screening, these materials showed adhesion to enamel and gold after 16 hours' immersion in water (Table IV). Both of these materials contained inorganic fillers, and neither of them used the typical spherical pearl polymer form as the organic portion of the powder.

The most promising reading in adhesion during the period being reported was obtained by combining a modified vinyl acetate containing acidic groups with polymethyl methacrylate and an aluminum oxide filler (see materials

TABLE I

ZINC OXYPHOSPHATE CEMENTS

COMMERCIAL PRODUCTS	5 MINUTES' SOAKING IN WATER AT ROOM TEMPERATURE			16 HOURS' SOAKING IN WATER AT ROOM TEMPERATURE		
	ENAMEL	DENTIN	GOLD	ENAMEL	DENTIN	GOLD
1	0	0	0	–	–	–
2	0	0	0	–	–	–

0 = No adhesion.
– = Not tested.

TABLE II

RESIN CEMENTS—PEARL POLYMERS

COMMERCIAL PRODUCT	5 MINUTES' SOAKING IN WATER AT ROOM TEMPERATURE			16 HOURS' SOAKING IN WATER AT ROOM TEMPERATURE		
	ENAMEL	DENTIN	GOLD	ENAMEL	DENTIN	GOLD
1. (Polymer plus filler)	**	0	****	0	–	0
2. (Polymer plus filler)	0	0	****	–	–	–
3. (Polymer plus filler)	*	0	****	0	–	0

0 = 0.
– = Not tested.
* = 1 – 5 pounds.
** = 6 – 10 pounds.
**** = 21 – ? pounds.

X-1 and X-3, Table V). Various formulations of this material were tested and found to maintain their adhesive qualities up to 1 week. Tests of percolation using bacteria indicated that after prolonged exposure to moisture at 37° C., the materials began to soften and swell, with resultant breakdown of the adhesive bond.

TABLE III

RESIN FILLING MATERIALS

COMMERCIAL PRODUCT	5 MINUTES' SOAKING IN WATER AT ROOM TEMPERATURE			16 HOURS' SOAKING IN WATER AT ROOM TEMPERATURE		
	ENAMEL	DENTIN	GOLD	ENAMEL	DENTIN	GOLD
1. (Polymer plus filler)	***	**	****	0	0	0
2. (Polymer plus filler)	**	0	0	0	–	–
3. (Polymer; no filler)	**	*	*	0	0	0
4. (Polymer; no filler)	*	*	0	0	0	0
5. (Polymer plus filler)	**	0	0	0	–	–
6. (Polymer plus filler)	*	0	0	0	–	0
7. (Polymer plus filler)	*	0	****	0	–	0
8. (Polymer; no filler)	***	0	0	0	0	0
9. (Polymer; no filler)	0	0	0	–	–	–

0 = 0.
– = Not tested.
* = 1 – 5 pounds.
** = 6 – 10 pounds.
*** = 11 – 20 pounds.
**** = 21 – ? pounds.

These data indicate that materials to be employed in tooth structure for their adhesive nature must not contain water-sensitive groups. It was found in general that plastics with a high water sorption are not useful as dental adhesives because of their tendency to swell and change dimension as the water sorption occurs. In the light of this work, it would be considered dangerous to assume that the water sorption value of the zinc oxyphosphate cements could be used as a standard for resin cements.

TABLE IV

RESIN CEMENTS—NONPEARL POLYMERS

COMMERCIAL PRODUCT	5 MINUTES' SOAKING IN WATER AT ROOM TEMPERATURE			16 HOURS' SOAKING IN WATER AT ROOM TEMPERATURE		
	ENAMEL	DENTIN	GOLD	ENAMEL	DENTIN	GOLD
1. (Polymer plus filler)	***	0	****	*	–	****
2. (Polymer plus filler)	**	0	****	*	–	***
3. (Polymer plus filler)	***	*	****	**	–	****

0 = 0.
– = Not tested.
* = 1 – 5 pounds.
** = 6 – 10 pounds.
*** = 11 – 20 pounds.
**** = 21 – ? pounds.

The epoxy resins screened by us were abandoned because of the difficulty of initiating polymerization at room temperature and because of published reports that they were carcinogenic.[7]

TESTS OF PERCOLATION USING BACTERIA

Percolation at the margins of plastic restorations as reported by Nelsen, Wolcott, and Paffenbarger[1] is a controversial subject in the field of dental

materials. The penetration of radiocalcium through the margins has been reported by other workers.[8] Seltzer* worked out a technic employing bacteria and bacterial spores. Using this technic, with modifications, a series of tests were planned to determine whether the fluid interchange in the space between plastic filling material and tooth structure would lead to the lodgment of bacteria. The objectives were: (1) to determine whether bacteria

TABLE V

SPECIAL AND EXPERIMENTAL POLYMER	5 MINUTES' SOAKING IN WATER AT ROOM TEMPERATURE			16 HOURS' SOAKING IN WATER AT ROOM TEMPERATURE		
	ENAMEL	DENTIN	GOLD	ENAMEL	DENTIN	GOLD
1. Epoxy Resin (Araldite CN-501, Ciba Co., Inc.)	0	0	-	-	-	-
2. Epoxy Resin (Exon RN34, Shell Chemical Co.)	0	0	-	-	-	-
3. Epoxy Resin (Potting compound 420)	0	0	-	-	-	-
4. Copolymer of styrene, acrylonitrile, and maleic anhydride	*	0	-	-	-	-
5. Copolymer of methacrylic acid and methyl methacrylate	0	0	-	-	-	-
6. Copolymer of vinyl carbazide coumarin, methacrylic acid, and methyl methacrylate	***	0	-	*	0	-
7. Polymethylmethacrylate (suspension polymer)	*	0	-	-	-	-
8. Polymethylmethacrylate (emulsion polymer)	0	0	-	-	-	-
9. Copolymer of methacrolein, methacrylic acid, and methyl methacrylate	**	0	**	*	0	0
10. Copolymer of styrene and maleic anhydride	***	0	**	0	0	0
11. Copolymer of vinyl acetate and maleic anhydride	0	0	-	-	-	-
12. Copolymer of methacrylic acid n-butyl methacrylate and methyl methacrylate	*	0	-	-	-	-
13. Copolymer of isobutyl methacrylate itaconic acid and methyl methacrylate	*	0	-	-	-	-
14. Styrene resin 820	***	-	-	0	-	-
15. Copolymer of acrylonitrile vinyl carbazole and methyl methacrylate	0	0	-	-	-	-
16. Copolymer of acrylonitrile coumarin and methyl methacrylate	0	0	-	-	-	-
17. Modified vinyl acetate containing acidic groups	****	**	****	**	0	****
18. X-3	***	*	****	0	0	****
19. X-1	****	*	****	**	0	****

0 = 0.
- = Not tested.
* = 1 – 5 pounds.
** = 6 – 10 pounds.
*** = 11 – 20 pounds.
**** = 21 – ? pounds.

*The technic used in this experiment was based on work reported by Samuel Seltzer, D.D.S., before the Philadelphia Section of the International Association for Dental Research, May 16, 1953.

will lodge in such spaces and the incidence of this occurrence; (2) to determine whether variations in environmental temperature affect the incidence of lodgment; (3) to determine whether lodgment can be prevented by the use of "adhesive" copolymers.

Technic.—Freshly extracted teeth were deposited in sterile 0.85 per cent NaCl and maintained at about 5° C. until used. Collection was continued until enough were available to begin the work. A Class 5 cavity, of reasonably constant external and internal dimensions, was prepared in the crown of each tooth. During preparation, with diamond disk and high-speed carbide burs, the teeth were frequently immersed in saline solution to prevent drying of the cavity area and the tooth. The cavity was prepared to fulfill all specifications required for plastic filling materials. The filling materials were mixed and placed in the cavity by the methods described by the manufacturers. The opening of the root canal at the apex of the root was sealed with a modified vinyl acetate–methyl methacrylate mixture. When the material had "set," the teeth were placed in sterile nutrient broth (Difco) for 24 hours, and then were polished according to accepted procedures. The junction of the filling material and enamel on each tooth was examined by use of a dissecting microscope; if any imperfections were found, the teeth were not used. The teeth were then divided into 7 groups, each being placed in a storage dish containing a 24-hour broth culture of *Pseudomonas aeruginosa.* (Plastic bags, perforated to allow circulation of culture, were employed to contain the teeth in the culture.)

The seven groups were treated as follows:

Group 1—maintained at 37° C. for the entire period of study.

Group 2—daily exposure to 5° C. for 15 seconds followed by 56° C. for 30 seconds.

Group 3—daily exposure to 56° C. for 15 seconds followed by 5° C. for 15 seconds.

Group 4—daily exposure to 5° C. for 15 seconds followed by 37° C. for 15 seconds, followed by 5° C. for 15 seconds.

Group 5—daily exposure to 56° C. for 15 seconds followed by 37° C. for 15 seconds, followed by 56° C. for 15 seconds.

Group 6—daily exposure to 5° C. for 5 seconds, followed by 37° C. at 5 seconds, followed by 56° C. for 5 seconds, and repeated once.

Group 7—daily exposure to 5° C. for 5 seconds, followed by 37° C. for 5 seconds, followed by 5° C. for 5 seconds, and repeated once.

At all other times, the storage dishes were kept at 37° C., the culture being renewed every 4 days.

These treatments were accomplished by dipping the plastic bag into 24-hour broth cultures of *Ps. aeruginosa* held at the various temperatures. The temperature changes had no effect on the viability of the bacteria in the cultures.

The first tests for the presence of viable bacteria beneath the filling materials were carried out after exposing the teeth for 14 days; the final tests were made after 30 days. The teeth were removed from the plastic bag, dried, and exposed to ultraviolet radiation for 7 minutes (General Electric germicidal lamp—G15T8—was mounted in a box so that a distance of 4 inches separated the middle of the lamp and the bottom of the box). The period of time employed had been determined by testing for the viability of *Ps. aeruginosa,* or any microorganisms, on the surface of the tooth or filling material. Cultures of material obtained from the surface of the tooth, or the surface of the filling material, were uniformly negative for growth. Thus, after ultraviolet radiation as specified, it was unlikely that bacteria would be carried into the cavity during removal of the filling material. The limited penetrating capacity of ultraviolet waves made it unlikely also that organisms within any space between filling material and tooth substance would be affected. A hot explorer was used to pierce the filling material and remove it from the cavity. A sterile bur was used to obtain grindings from the surfaces of the cavity. The grindings were collected and transferred to sterile broth by means of sterile paper points (type used in endodontics) moistened in sterile broth and incubated at 37° C. for at least 7 days. Cultures of samples showed either growth and chromogenesis (green-blue color) or no growth. If there was doubt about chromogenesis, a transfer was made to sterile nutrient agar (Difco) and the subculture incubated. Appearance of color (as previously) in the medium was recorded as positive for *Ps. aeruginosa.* Absence of such characteristics was recorded as negative for the test bacterium.

The sampling procedure described was tested to determine the lowest numbers of the test bacteria required in the grindings to obtain a positive culture. It was found that the method would be positive for at least 200 bacteria in the cavity before grindings were obtained.

Materials Tested.—

1. Control—A standard resin filling material* was purchased from a local dealer and used as a basis of comparison with the experimental materials.

2. Three experimental materials which had shown promise in adhesion testing were tested as shown in Tables VII and VIII.

(a) X-1—A mixture of 50 per cent high-grade methyl methacrylate and a modified vinyl acetate containing acidic groups.

(b) X-2—The same formula as X-1 with the addition of 20 per cent finely divided aluminum oxide as a filler.

(c) X-3—Methyl methacrylate containing 10 per cent of the modified vinyl acetate and 25 per cent aluminum oxide filler.

RESULTS

The data presented in Table VI indicate that the test bacteria lodge in a space between this filling material and tooth structure. The sampling at

*Since this test, the manufacturer has brought out an improved material, not yet submitted to this type of test.

14 days was performed to determine preliminarily whether there were differences in the various kinds of exposures. Only 2 teeth were sampled out of each group and mostly negative cultures were obtained (3/14). Rather than sampling from all other teeth in those groups where cultures were positive, all tests were continued until 30 days had elapsed.

TABLE VI

RESULTS FROM SAMPLES OF TEETH FILLED WITH COMMERCIAL RESIN FILLING MATERIAL

	1	2	3	4	5	6	7
	14 DAYS			30 DAYS			
GROUPS*	†	0	† † and 0	†	0	† † and 0	‡
1	0	2	0/2	5	1	5/6	5/6
2	0	2	0/2	2	3	2/5	2/5
3	0	2	0/2	-	-	-	-
4	0	2	0/2	4	2	4/6	4/6
5	1	1	1/2	2	2	2/4	3/5
6	2	0	2/2	2	2	2/4	4/6
7	0	2	0/2	2	3	2/5	2/5
			3/14			17/30	20/33

0 – No growth.
*See text for varying exposures of groups of teeth.
†Growth and chromogenesis indicating presence of *Pseudomonas aeruginosa.*
‡ (14 days) plus † (30 days) / ‡ (14 days) plus † and 0 (30 days)

Columns 3, 4, and 5 (Table VI) show the results of cultures of samples from teeth in each group. There were 5 teeth in Group 1 (teeth containing the filling material were kept at 37° C. for 30 days) from which samples contained the test bacterium; 4 teeth from Group 2; and 2 teeth from each of the other groups tested. The container holding the teeth in Group 3 was broken accidentally and the teeth lost for the cultural tests. Although the numbers of teeth tested are small, it appears that the changes in temperature of exposure did not materially affect the results. A greater number of positive cultures were obtained from teeth kept at 37° C. for the period (Group 1) than from any other group. Since the exposure to varying temperatures consisted of a relatively short interim of time (not more than 1 minute in 24 hours) a summary (see Column 6) of the results shows that in over one-half of the samples (17/30) the test bacterium could be isolated from the cavities.

TABLE VII

RESULTS FROM SAMPLES OF TEETH FILLED WITH MATERIAL X-1

GROUPS	*	0	* AND 0
1	0	2	0/2
2	1	1	1/2
3	1	1	1/2
4	2	0	2/2
5	2	0	2/2
6	2	0	2/2
7	0	2	0/2
			8/14

0 – No growth.
*Growth.

TABLE VIII

RESULTS FROM SAMPLES OF TEETH FILLED WITH MATERIALS X-2 AND X-3

GROUPS	X-2			X-3		
	*	0	* AND 0	*	0	* AND 0
1	5	4	5/9	5	4	5/9
2	3	1	3/4	3	1	3/4
			8/13			8/13

0 – No growth.
*Growth.

Column 7 indicates a summary of the cultural tests of samples from the teeth. The ratios are obtained by combining the number of *positive* cultures in the 14-day sampling with the number of *positive and negative* cultures from the 30-day test. It is assumed that the growth of the test bacterium in cultures of samples from teeth at 14 days also would have been found at 30 days.

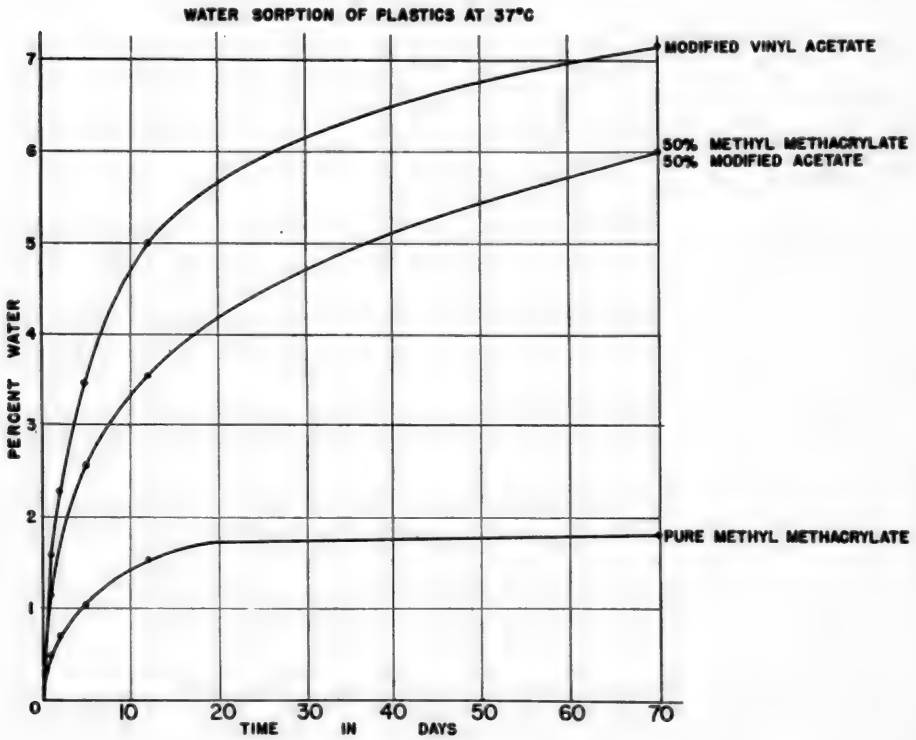

Fig. 4.

Tooth samples found negative at 14 days are not included, because it is impossible to predict whether samples from these teeth would have contained the test bacterium after further exposure. Combination of the findings in this manner indicates that the test bacterium could be isolated from the cavities of over one-half the teeth, by the sampling method, from all groups except

2 and 7. The small numbers of teeth employed make it difficult to determine whether any of the observed differences are real. If the temperature alterations are set aside, the results indicate that over one-half the samples (20/33) contained the test bacterium.

Material X-1 was used to fill prepared cavities in 14 teeth. The teeth were divided into 7 groups and exposed to the same conditions as seen in Table VI. The findings, after culturing samples from these teeth, are presented in Table VII. The test was conducted only 14 days because of failure of the filling material. (It was observed that the material was no longer level with tooth structure at the margins, but had apparently swollen and extended outward. This was probably due to excessive water absorption.)

Cultures of all samples from Groups 4, 5, and 6 were positive for the test bacterium; 1 sample out of 2 from Groups 2 and 3 was positive; the samples from Groups 1 and 7 either did not contain the test bacterium or not enough bacteria were present to initiate growth in the culture medium.

Results of tests on materials X-2 and X-3 are presented in Table VIII. The daily exposure to temperature alteration appears to have affected the proportion of positive cultures. Samples from teeth stored at 37° C. were positive in 5 to 9 teeth in both instances, whereas exposure to 5° C. for 15 seconds daily resulted in 3 of 4 samples being positive for the test bacterium.

It is apparent that a test bacterium will lodge between a plastic filling material and tooth substance, even though our methods may not be as accurate as those using a radioactive chemical. The effects of temperature appear to depend on the type of plastic filling material employed. Modification of polymers to obtain adhesive properties may increase the water sensitivity of the filling material (Fig. 4). The latter results in a degradation of the adhesive bond in a short time of exposure to water.

SUMMARY

1. More than 5,500 individual tests for the adhesion of various materials to tooth structure were conducted.

2. There is a difference in adhesive qualities when materials are used against enamel as compared to dentin.

3. The behavior of adhesives when tested on dry teeth has no direct bearing on the potential adhesion of the same material in the presence of moisture.

4. Fillers show some promise toward improving adhesion by better wetting of tooth surface and a potential decrease in the thermal coefficient of expansion of resins, but are not the final answer to the problems.

5. No products presently available for testing consistently maintain adhesion after prolonged water immersion.

6. High water sorption destroys the adhesive values of resins.

7. The data indicate that the test bacteria can lodge between the filling materials employed and tooth substance, when teeth are maintained in the culture at 37° C. Effects of temperature variation could not be evaluated accurately.

8. Addition of a filler to a copolymer did not reduce the chances for lodgment of the bacteria.

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DIRECT RESIN FILLING MATERIALS: COEFFICIENT OF THERMAL EXPANSION AND WATER SORPTION OF POLYMETHYL METHACRYLATE*

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INTRODUCTION

DIRECT resin filling materials expand about 7 times as much as the natural tooth structure (the linear coefficient of thermal expansion for methyl methacrylate resin was calculated by Souder and Peters[1] as 0.000081 per °C., while the similar value for tooth structure was stated to be approximately 0.000011 per °C.). In addition, Smith and Schoonover[2] described polymerization shrinkage amounting to 6 to 8 per cent by volume in the case of resin filling materials employing methyl methacrylate as the chief constituent of the liquid monomer. Due to poor adhesion of the resin filling material to the walls of the tooth cavity, polymerization shrinkage may create a crevice between the cavity walls and the resin filling. The much higher coefficient of thermal contraction of the resin material as compared to the tooth structure causes enlargement of this crevice during chilling of the tooth. When the temperature rises, the crevice narrows down. These temperature fluctuations may lead to the phenomenon of percolation as described by Nelsen, Wolcott, and Paffenbarger[3] as well as lodgment of debris and bacteria[4, 5] in the space between the filling and the cavity walls. This suggests that an appreciable decrease in the coefficient of thermal expansion of the resin might be a significant gain in reducing or preventing the marginal percolations and the lodgment of debris and bacteria.

It is generally believed that by incorporating suitable inorganic fillers in the resins, the coefficient of thermal expansion or contraction can be decreased; this is found to be true in the case of phenol-formaldehyde and other similar resins. However, to our knowledge no such data have been published for acrylic resins. According to Glenn,[6] the coefficient of expansion of acrylic resins loaded with different amounts of various inorganic fillers did not follow a definite pattern as the amount of filler was increased. The same investigator[6] reported that in some cases there was even a slight increase in the coefficient of expansion of the filled resin.

There are a large number of polymeric materials which show good temporary adhesion to natural tooth structure. Rose, Lal, Williams, and Falcetti[5] have shown that adhesion may be gradually destroyed as absorption of water progresses, probably due to the considerable increase in weight and volume.

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In the case of acrylic resins, several investigators[7, 8] have shown that the polymerized resin definitely gains in weight when placed in water. According to Emerson,[9] no observable volumetric change resulted. Schoonover[10] reported a minor increase in volume. Recently, linear expansion due to water sorption for a number of commercially available dental products has been reported by Smith and Schoonover[2] and earlier by Skinner and Cooper.[11] These studies[2] indicate that if no strain is present, the direct resin filling material expands equally in all directions as a result of water sorption. A linear expansion of 0.3 to 0.5 per cent or a volume expansion of 1.0 to 1.5 per cent was found.

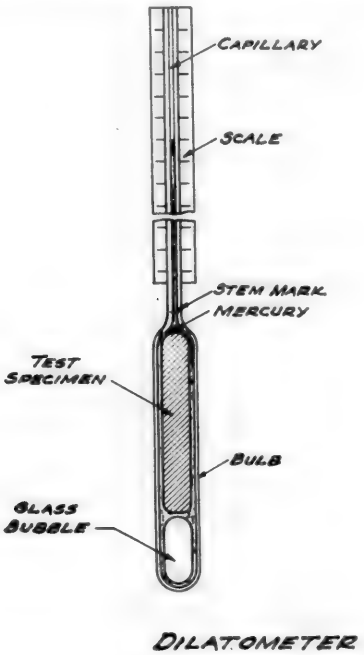

Fig. 1.

Water sorption by acrylic resin and consequent volumetric increase is probably one of the causes of breakdown of marginal adaptation of resin to tooth. It is possible that a resin with a lower water sorption than polymethyl methacrylate may perform better. This paper, therefore, reports a series of studies of water sorption of polymethyl methacrylate as influenced by: (a) the addition of fillers, (b) cross-linking the resin, and (c) change of pH of the water.

EXPERIMENTAL PROCEDURE AND RESULTS

The procedure used for determining coefficient of expansion was a slight modification of the A.S.T.M. Method.[12] Two parts by weight of the polymer containing 2 per cent benzoyl peroxide were mixed with 1 part of methyl methacrylate monomer activated by an aromatic tertiary amine. The dough

was polymerized under pressure into rods about 10 cm. long and an area of cross section of about 0.4 sq. cm. A simple dilatometer was used to determine the volumetric change resulting from the expansion of the experimental resin sample when the temperature was raised from 0° C. temperature to 25° C.; displacement of mercury in the capillary tube was accurately measured. For experimental convenience, a constriction was placed at the upper end of the bulb to prevent the resin sample from blocking the lower end of the capillary tube (Fig. 1). Five different inorganic fillers (screened through 200 mesh Tyler screen) were used. The filler was mixed with fine, ground polymer containing benzoyl peroxide and the mixture was rolled in a small ball-mill for sixteen hours. Five different specimens were prepared for each filler by varying the composition of the powder so that the final product would contain 10, 20, 30, 40, and 50 per cent of filler. The fillers used consisted of alumina, silica, boron phosphate, calcium carbonate, and zinc oxide. The results obtained are shown in Tables I and II and curves are drawn in Figs. 2 and 3.

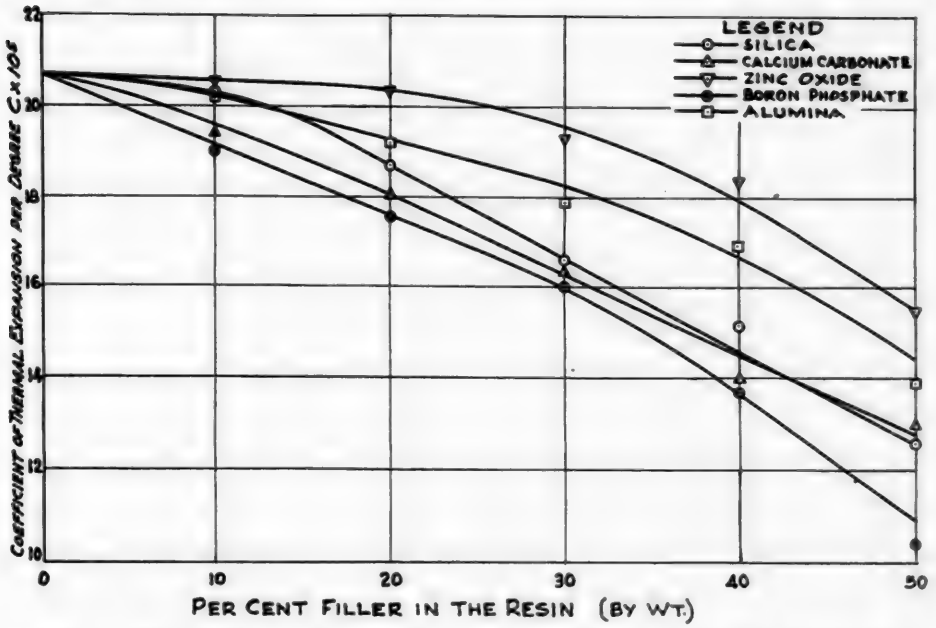

Fig. 2.—Effect of fillers on the coefficient of thermal expansion of polymethyl methacrylate.

In Fig. 2, the volumetric coefficient of thermal expansion of various samples is plotted against the weight of the filler in the final resin; similarly, in Fig. 3, the change in the volumetric coefficient of expansion is plotted against the weight of the filler in the final resin. The results show that the coefficient of expansion decreases as the amount of the filler in the final resin increases. In no case did we observe any increase in the coefficient of expansion when any of the fillers tested were added.

TABLE I

EFFECT OF FILLERS ON THE COEFFICIENT OF THERMAL EXPANSION OF POLYMETHYL METHACRYLATE

WEIGHT PER CENT OF FILLER IN THE POLYMETHYL METHACRYLATE RESIN	COEFFICIENT OF VOLUMETRIC EXPANSION X10⁵				
	SILICA	CALCIUM CARBONATE	ZINC OXIDE	BORON PHOSPHATE	ALUMINA
50	12.6	13.0	15.5	10.4	13.9
40	15.2	14.0	18.4	13.7	16.9
30	16.6	16.3	19.3	16.0	17.9
20	18.7	18.1	20.4	17.6	19.2
10	20.3	19.4	20.6	19.0	20.2
0	20.7	20.7	20.7	20.7	20.7

TABLE II

PER CENT DECREASE IN THE COEFFICIENT OF THERMAL EXPANSION X10⁵ OF POLYMETHYL METHACRYLATE

WEIGHT PER CENT OF FILLER IN THE POLYMETHYL METHACRYLATE RESIN	PER CENT DECREASE				
	SILICA	CALCIUM CARBONATE	ZINC OXIDE	BORON PHOSPHATE	ALUMINA
50	39.6	33.8	25.6	49.9	32.2
40	26.5	32.4	12.0	33.8	18.3
30	19.8	22.8	7.0	22.7	13.5
20	10.0	13.0	2.0	14.0	7.3
10	1.9	6.3	0.5	1.9	2.4

FIG. 3

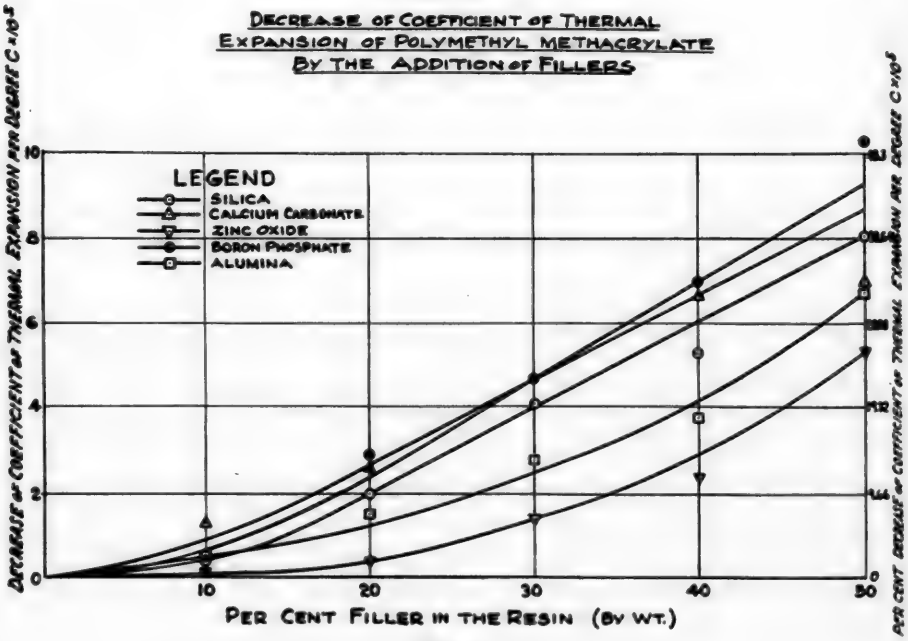

Fig. 3.

The results indicate that at lower concentrations of the filler in the resin, most of the fillers studied behave alike; however, at higher concentration, some differences are noticeable.

The effect of fillers on the water sorption of the resin sample is shown in Fig. 4. Samples used were of the same size as used in the determination of the coefficient of thermal expansion. Generally speaking, fillers decrease the water sorption of the resin. In the case of some fillers, decrease in weight of the specimens is noted after the initial increase; this may be explained by the slight solubility of the fillers used in these resin samples.

FIG. 4

EFFECT OF FILLERS IN WATER SORPTION OF POLYMETHYL METHACRYLATE

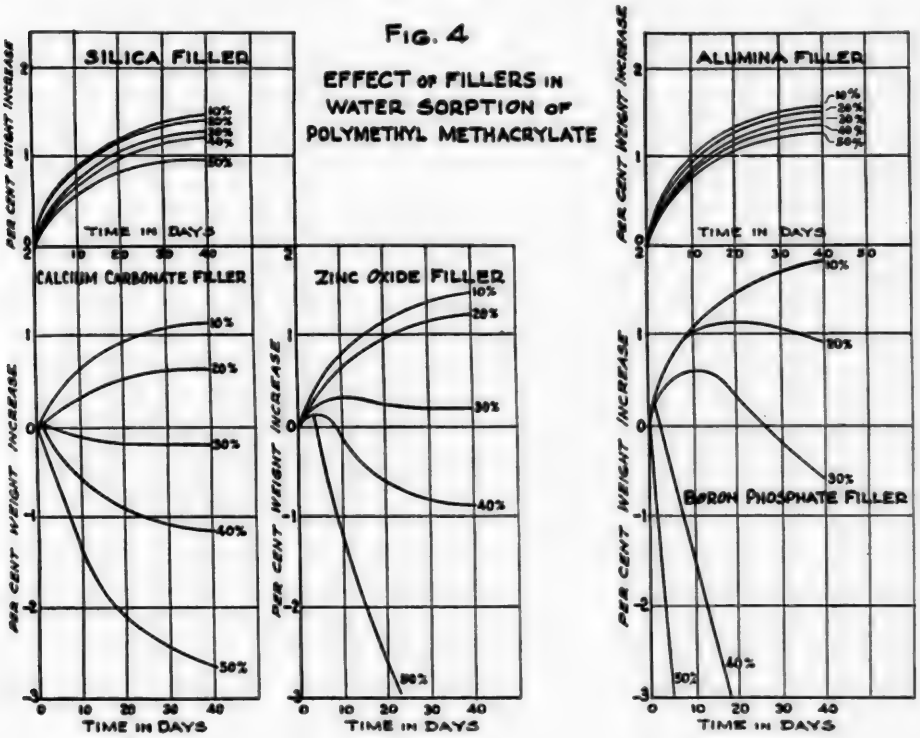

Fig. 4.

The influence of cross-linking and pH on the water sorption of polymethyl methacrylate was studied. Samples 1 inch by 1 by ⅛ inch in size were polymerized under pressure from 2 parts by weight of polymer powder containing 2 per cent peroxide catalyst and 1 part of monomer containing 0.5 per cent N, N-dimethyl-p-toluidine. Three monomer compositions were used: one was a standard high-grade methyl methacrylate and the other two contained 20 and 40 per cent by weight of ethylene glycol dimethacrylate. Buffered solutions of pH 3,5,7, and 9.2 at 37° C. were used for water sorption studies. Water sorption was studied at a given pH for materials with varying degree of cross-

linking. Samples were dried well in folds of cloth prior to weighing. The results are shown in Figs. 5 and 6 by plotting per cent increase in weight against time (number of days). It is apparent that water sorption of polymethyl methacrylate is not significantly changed by varying either the pH or the degree of cross-linking. Water sorption tends to approach a maximum value of about 2 per cent in 4 weeks' time.

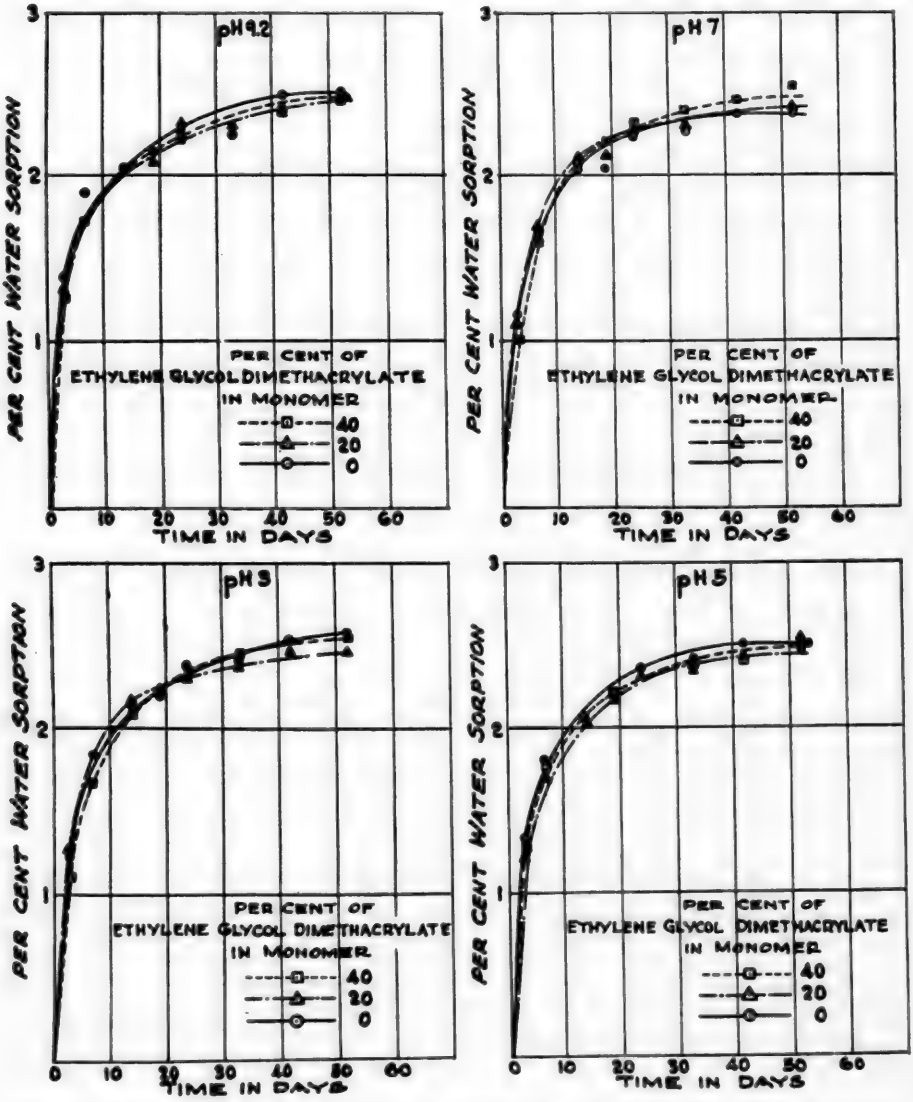

Fig. 5.—Effect of cross-linking on the water sorption of polymethyl methacrylate at different pH.

SUMMARY

The data obtained indicate that fillers can be used with advantage to reduce the coefficient of thermal expansion of acrylic resins. Proper mixing of the polymer powder and the inorganic filler is of considerable importance since improper mixing might lead to poor wetting and give erratic results. The various fillers studied by us do not materially differ one from the other at low concentrations in so far as their influence on coefficient of thermal expansion is concerned; however, at high concentrations of the filler in the resin, some

FIG. 6

EFFECT OF pH ON WATER SORPTION OF CROSS-LINKED AND UNCROSS-LINKED POLYMETHYL METHACRYLATE

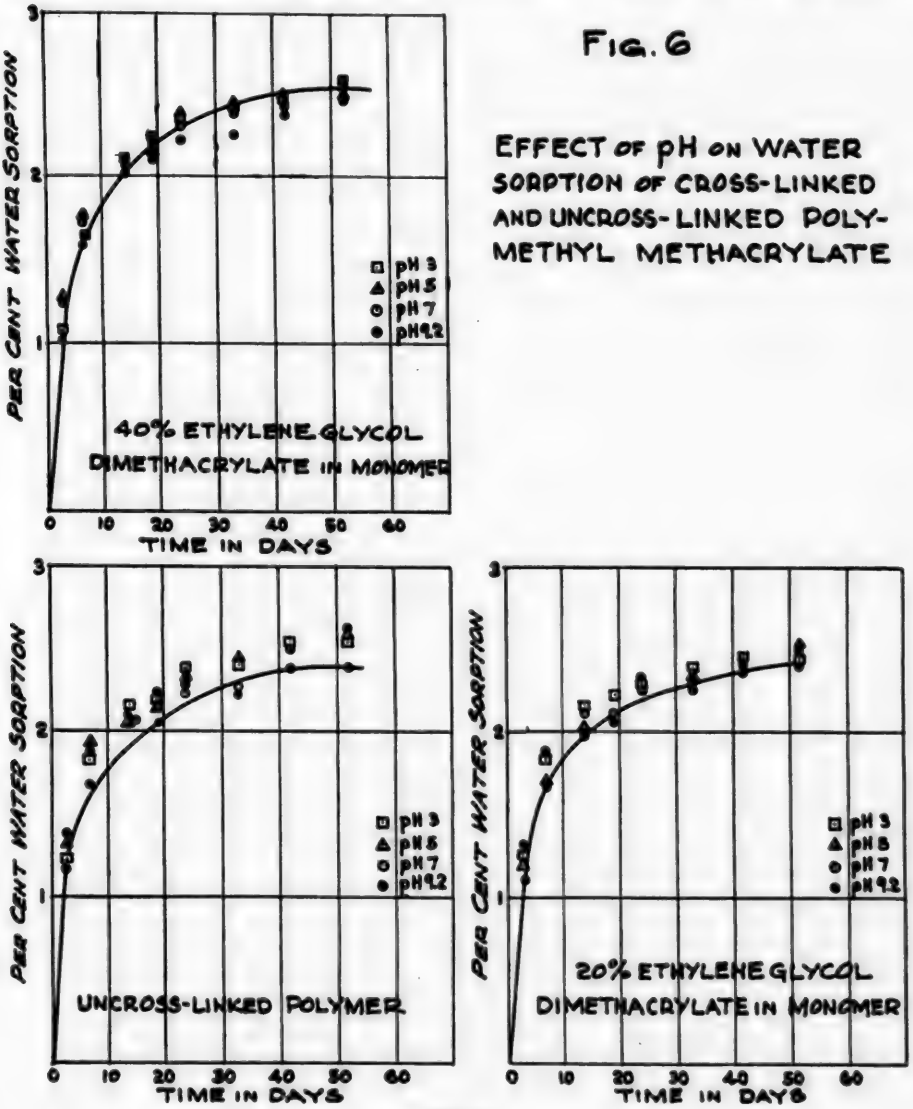

Fig. 6.

differences are noticeable. The choice of a suitable filler should be made after taking into consideration the optical properties of the resultant resin; in this connection the index of refraction of the filler and its particle size distribution should be given adequate attention.

Studies of the increase in weight of direct filling resins in water also point to the beneficial effect of fillers. Fillers which are water-soluble in any appreciable degree are likely to cause the resin to become porous in character after a period of time; their value is doubtful. In the initial stage, most of the fillers studied reduced the water sorption of the resulting resin to about the same extent, regardless of solubility.

Addition of cross-linking materials to the acrylic resin did not significantly change its water absorption characteristics.

CONCLUSIONS

1. The coefficient of thermal expansion of polymethyl methacrylate decreases with the addition of various inorganic fillers.

2. The greater the amount of the filler added to polymethyl methacrylate the greater is the reduction in the coefficient of thermal expansion.

3. Weight increase due to water sorption of polymethyl methacrylate is reduced by the addition of various inorganic fillers; higher percentages of filler added produce greater reductions in the weight gain.

4. Addition of cross-linking material to the polymethyl methacrylate does not significantly change the water sorption properties of the resin.

5. Change of pH has no apparent effect on the water sorption of polymethyl methacrylate.

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MEASUREMENT OF THE ELECTRIC CONDUCTIVITY OF DENTAL CEMENT

III. Effect of Increased Contact Area and Thickness; Values for Resin, Calcium Hydroxide, Zinc Oxide–Eugenol

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A DISCUSSION of the instrumentation, circuit design, and cell construction which have been developed for accurately measuring the electric conductivity of dental cements has been presented in a previous publication.[1] A few preliminary experiments were summarized in that report, showing the resistance pattern of zinc phosphate cement upon aging. In those tests the experimental cell was carefully sealed to prevent loss or gain in moisture. In a later publication[2] several problems were studied, including the effect of moisture on the electric conductivity of zinc phosphate cement. The cement margin was exposed to normal saline solution and to distilled water. In both media the insulating ability was markedly reduced.

The purpose of this investigation was to study the effect of contact area when the margin was exposed, to evaluate the effect of thickness of the cement layer, to determine the effect of substituting gold for copper in the experimental cell, and to include measurements on the electric conductivity of other commonly used cementing materials.

INCREASED CONTACT AREA

In previous research[2] the effect of contact area on the conductivity of zinc phosphate cement, when maintained in a dry condition, was studied. The values which were obtained on large cells are plotted in Fig. 1. Additional tests were now made exposing the cement margin to normal saline solution. The thickness of the cement was retained at 1 mm., but the contact area of the cement and metal was increased from 38.45 sq. mm. to 290.00 sq. mm. A total of 10 cells was measured, and the average resistance of the cells under these conditions is plotted in Fig. 1.

Exposure of the margin to moisture followed the same pattern found with the small cells and reduced the insulating ability of the zinc phosphate cement. The loss in resistance was not as great, however. With the small cells the resistance of the dry cement reached values over 1,000 megohms in 1,200 hours

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while the dry cells with increased contact area, although they did show a progressive increase in resistance, had an average value of only 3.6 megohms at that time interval. The magnitude of resistance for both large and small cells when exposed to saline solution was comparable. These data seem to indicate that the variable, increased contact area is much less effective in altering the electric conductivity when the cement is wet than when it is maintained dry.

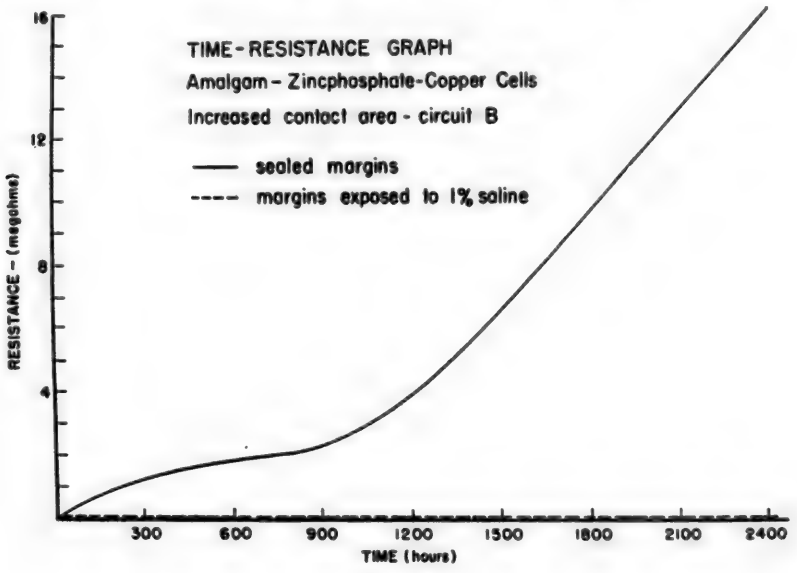

Fig. 1.

INCREASED THICKNESS

One of the suggested clinical methods for minimizing the postoperative pain associated with galvanic currents is to increase the thickness of the cement base. Thus, a series of tests was made in which the conductivity was correlated with the thickness of the cement layer. Cells were constructed increasing the cement thickness from 1 to 2 mm. One group of 8 cells was sealed while another group of 6 cells was prepared with the margins exposed to normal saline solution. One cell was run as a control, omitting the cement entirely between the copper and amalgam plugs.

Those cells which were sealed produced the typical pattern of increasing resistance with time (Fig. 2). For comparative purposes, the resistance for the normal cells, 1 mm. thickness, is also shown. It is evident that the resistance did increase at a somewhat greater rate in the thicker cement cells, but in both cases, the resistance exceeded 1,000 megohms and indicated adequate insulating ability for either thickness when the cement margin was sealed.

The cells with margins exposed to saline solution also behaved in the conventional manner (Fig. 2). The moisture resulted in greatly lowered resist-

ance, less than 0.1 megohm, in all cases. The resistance measured on the control cell was essentially the same as the values that were obtained on the specimens where the margin was exposed to saline solution. Thus, for all practical purposes, it appears that the zinc phosphate cement, even though the thickness was increased, added little to the electric insulation when the margin was exposed to moisture. This observation has been confirmed clinically in a recent study by Nachlin.[3] He found that reducing the mass of metal and increasing the thickness of the layer of cement did not eliminate the pain which he associated with galvanic currents.

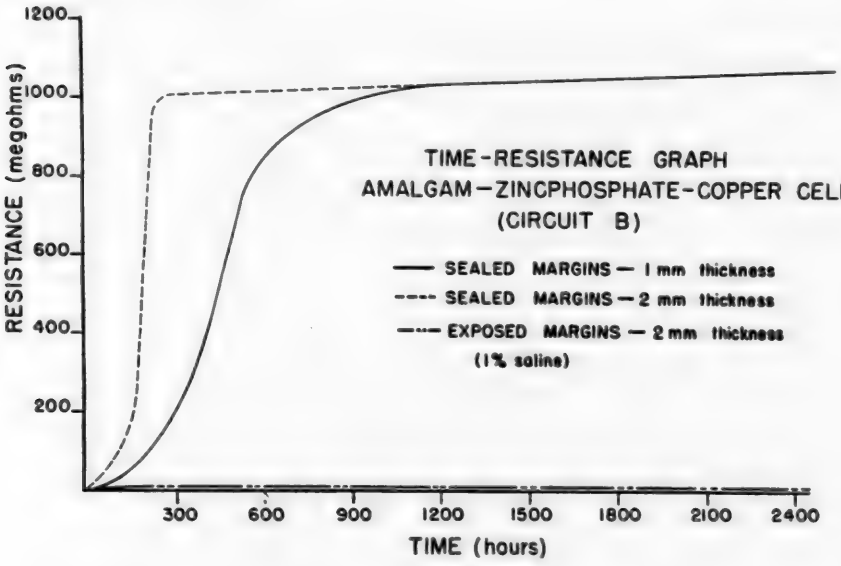

Fig. 2.

AMALGAM–ZINC PHOSPHATE–GOLD CELLS

It was evident from all the previous tests that the resistance of zinc phosphate cement was influenced markedly by the environment to which the cement margin was exposed. The resistance of the cement was always high when the cell was sealed against moisture, but dropped greatly upon exposure to saline solution or distilled water. The amount of moisture to which the cement is subjected clinically, either from the dentin itself or by exposure to saliva at the margin, is, of course, unknown. However, it was felt that since moisture did play such a vital role in the electric resistance, a different type of cell could be designed that would simulate the clinical conditions somewhat more closely. Thus, a cell was constructed which basically was comparable to the cemented clinical restoration.

A cavity preparation similar to a full-cast crown was prepared in a Lucite mold (Fig. 3). An amalgam plug was packed in the center of the mold with

a 1 mm. recession at the top for cement (simulating the cement base). A casting was made for this preparation, and it was cemented onto the prepared surface with zinc phosphate cement (Fig. 3). Electric connections for the cell were then made to the gold and to the amalgam. This type of cell not only more closely resembled the type of margin present in the clinical inlay or crown, but also made possible the study of a gold-amalgam cell as compared to the copper-amalgam cell previously used.

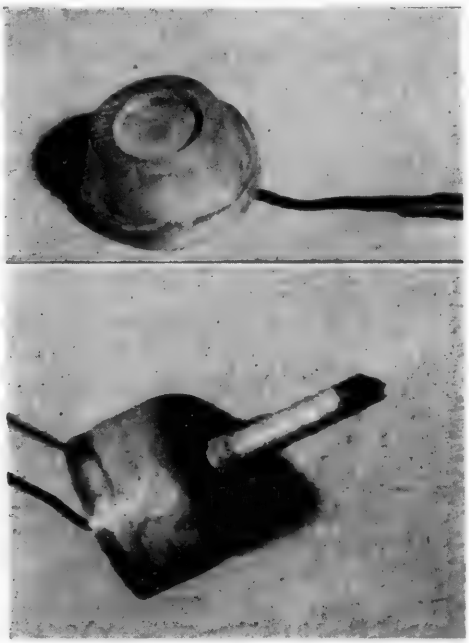

Fig. 3.—Cavity preparation prepared in Lucite mold (top). Casting cemented onto prepared base with electrical connection (bottom).

Two groups of cells were studied. Four cells were run with the margin sealed with compound while 5 had the margins exposed to saline solution. The results were comparable to previous tests, and the average resistances have been plotted in Fig. 4. When sealed, the cells increased in resistance with time. Although the values reached at any given time interval were less than those obtained with the flat amalgam–zinc phosphate–copper cells used previously, the magnitude still indicated adequate insulating ability.

When the margin was exposed to normal saline solution, the resistance was again low, the average being less than 1 megohm. These values were only slightly higher than those of the amalgam–zinc phosphate–copper cells used in previous tests.

In general, with this cell, the cement resistance under dry conditions reached values which indicated adequate insulating ability; however, when

exposed to saline solution, the resistance of the cement was low and was comparable to the values obtained in the previous tests. The use of gold in place of copper did not alter the resistance pattern of the cementing material.

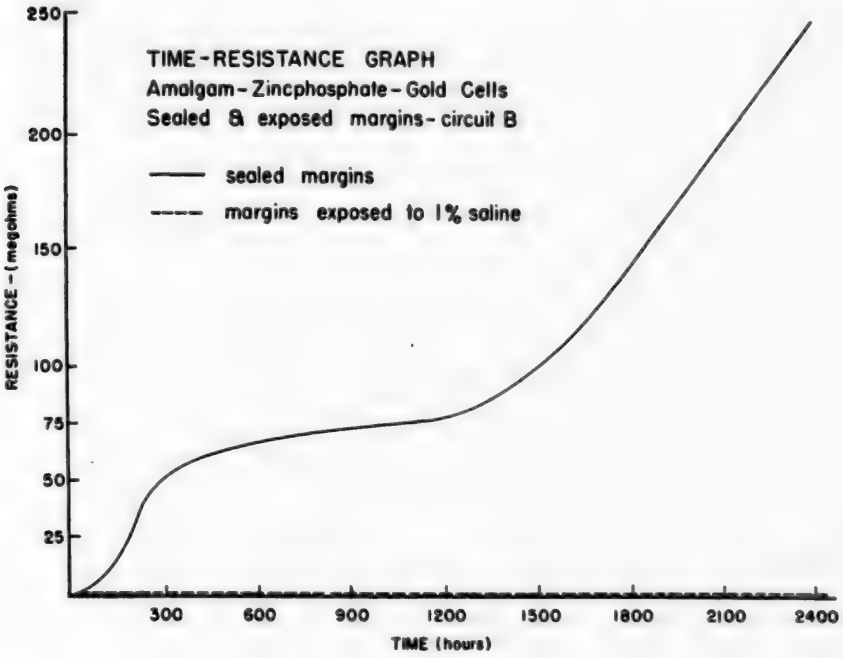

Fig. 4.

CONDUCTIVITY OF CALCIUM HYDROXIDE, RESIN, ZINC OXIDE–EUGENOL

Materials other than zinc phosphate cement are used as luting agents for cement bases and for pulpotomies. It was the purpose of the next series of tests to study the electric insulating properties of some of the commonly used materials.

Calcium hydroxide paste was employed as a separating medium between the amalgam and copper plugs, and the cells were constructed with the thickness of the material maintained at 1 mm. Three groups of cells were used. One group had the margins sealed and was run on an open circuit,[1] while a second group was sealed but was run on a closed circuit.[1] The third group was run on closed circuit, but the margins were exposed to normal saline solution.

There was very little difference in the conductivity between the 3 groups (Table I). The sealed cells on open circuit did have somewhat higher values of resistance and showed a steady increase with time. Whether these cells will continue to increase in resistance over a longer period of time remains to be seen. Since insulation is of primary importance in the period immediately after insertion of the restoration, probably there is little value to increased resistance

TABLE I

TIME OF READING AFTER ASSEMBLY (HR.)	CELL NUMBER AND RESISTANCE IN MEGOHMS																
	SEALED—OPEN CIRCUIT						SEALED—CLOSED CIRCUIT					EXPOSED—CLOSED CIRCUIT					
	I	II	III	IV	V	AV.	I	II	III	IV	AV.	I	II	III	IV	V	AV.
1	0.065	0.185	0.065	0.107	0.085	0.101	0.030	0.030	0.050	0.030	0.035	0.150	0.030	0.022	0.150	0.032	0.077
2							0.030	0.030	0.060	0.030	0.037						
4	0.150	0.295	0.173	0.062	0.085	0.153	0.080	0.050	0.075	0.050	0.054	0.150	0.065	0.045	0.023	0.075	0.072
8	0.107	0.400	0.150	0.107	0.270	0.207	0.040	0.075	0.050	0.030	0.059	0.020	0.065	0.095	0.065	0.075	0.064
16	0.128	0.470	0.085	0.118	0.360	0.232	0.040	0.148	0.040	0.030	0.065	0.065	0.065	0.085	0.063	0.060	0.068
32	0.140	0.307	0.207	0.198	0.360	0.242	0.050	0.198	0.096	0.050	0.096	0.095	0.085	0.150	0.235	0.085	0.130
64	0.195	0.360	0.270	0.345	0.245	0.283	0.075	0.208	0.160	0.065	0.127	0.095	0.075	0.150	0.150	0.105	0.115
128	0.175	0.327	0.270	0.390	0.235	0.279	0.060	0.250	0.160	0.160	0.158	0.100	0.088	0.162	0.135	0.100	0.117
256	0.175	0.270	0.270	0.515	0.245	0.295	0.040	0.060	0.120	0.100	0.080	0.175	0.105	0.175	0.127	0.175	0.151
384	0.175	0.295	0.270	0.555	0.415	0.242	0.040	0.060	0.165	0.165	0.107	0.300	0.255	0.105	0.175	0.250	0.217
512	0.185	0.305	0.270	0.575	0.430	0.253	0.040	0.050	0.230	0.230	0.137	0.395	0.580	0.255	0.120	0.165	0.303
768	0.198	0.385	0.333	0.670	0.360	0.389	0.040	0.040	0.120	0.190	0.098	0.350	0.098	0.120	0.140	0.165	0.175
1,200	0.183	0.400	0.545	0.670	0.430	0.446	0.300	0.037	0.275	0.370	0.245	0.098	0.058	0.098	0.140	0.098	0.098
2,400	0.230	0.770	0.507	0.705	0.995	0.621	0.322	0.300	0.275	0.165	0.965	0.230	0.230	0.140	0.205	0.080	0.177

after 3 months. From these data, then, it is evident that calcium hydroxide does not offer much resistance to electric energy, either when maintained in a dry condition or when the margin is exposed to saline solution.

The use of an acrylic resin cement as an insulating medium was also investigated under both the dry and the wet conditions. Fourteen cells were tested. It was found that when the cell was sealed the resistance was always over 1,000 megohms initially (1 hour) and remained at that magnitude for the entire period of the test, 2,760 hours. Even when the margins were exposed to saline solution, the resistance pattern was unaltered, measuring more than 1,000 megohms from the initial reading. All the other materials previously tested had shown a marked reduction in resistance when the margin was exposed; thus, it appeared that either the resin was not influenced by moisture, or a better marginal seal was being obtained with this material, and it prevented penetration of the saline solution. The latter explanation seemed to be the most logical since Sausen, Armstrong, and Simon[4] have shown by means of radioactive materials that there is no penetration into the mass of the acrylic material. As the molds themselves were Lucite, the resin cement might have actually bonded to the Lucite. Such a condition, of course, is not present in the mouth, where the resin would be in apposition to tooth structure. Since it is the primary purpose of this study to determine basic values of electric conductivity for these materials, it was felt that a different type of test might be devised where the marginal seal of the cement would not be a possible variable.

A cell was constructed as diagramed in Fig. 5. An amalgam plug (*C*) was surrounded completely by the insulating material (*D*), resin cement. The container for the cement, which served as the other electric connection, was a copper band (*A*). The outer surface of the material (*E*) was then exposed to a normal saline solution. Thus, in this cell, the moisture had to be absorbed by the resin in order for it to alter the conductivity. If the resin did not absorb water, then there could be no completed electric circuit between the copper band and the amalgam plug.

The results obtained with this particular cell verified previous tests made with the other types of cells. The resistance remained high throughout the immersion period; thus, resin cement, either wet or dry, gave evidence of being an excellent insulating medium.

Cells of the type shown in Fig. 5 were also utilized to study the conductivity of zinc phosphate cement. The results with this cell corroborated the data that had been obtained previously with this material. When the cement was maintained in a dry condition, the resistance was high, but when exposed to saline solution, the resistance values decreased markedly. Whether the cement became conductive as a result of uniform absorption of moisture, or by penetration of the saline solution through cracks in the zinc phosphate cement, is as yet undetermined. Preliminary studies seem to indicate the latter.

TABLE II

TIME OF READING AFTER ASSEMBLY (HR.)	CELL NUMBER AND RESISTANCE IN MEGOHMS													
	SEALED 1 MM.	CIRCUIT B—EXPOSED 2 MM. THICKNESS				CIRCUIT B—MARGINS EXPOSED—1 PER CENT SALINE SOLUTION—1 MM.								
	I	II	III	IV	AV. II, III, IV	I	II	III	IV	V	VI	VII	VIII	AV.
1		12.2	12.6	9.32	11.4	5.39	6.42	5.82	5.29					
2	6.29									6.04	10.3	6.44	6.04	7.21
4	7.58	16.7	12.9	9.32	12.9	6.05	6.29	5.39	4.49	5.66	7.42	6.97	6.41	6.08
8	10.1	16.7	11.2	7.43	11.8	5.60	5.71	5.71	2.16	5.19	7.42	6.82	6.41	5.63
15	8.68	358	201	7.77	160	6.97	6.05	5.82	1.08	1,000	1,000	5.09	5.00	129
30	10.9	1,000	1,000	1,000	1,000	1,000	758	1,000	1,000	1,000	1,000	1,000	1,000	1,000
60	5.38	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
120	4.48	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
256	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
384	1,000									1,000	1,000	1,000	5.05	1,000
512	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	5.77	1,000
768	1,000	80.8	1,000	1,000	1,000	404	1,000	1,000	1,000	1,000	1,000	1,000	3.50	1,000
1,200	1,000	310	1,000	1,000	1,000	560	1,000	1,000	1,000	1,000	1,000	1,000	3.50	1,000
2,400	1,000	40.4	8.52	1,000		668	1,000	1,000	19.1	1,000	1,000	1,000	0.295	1,000

Cells were also constructed in which the insulating medium was zinc oxide–eugenol paste. Three groups were tested, one with sealed margins and a 1 mm. thickness of insulating material, one with exposed margins and a 1 mm. cement layer, and a third group with exposed margins and a 2 mm. insulating layer of cement.

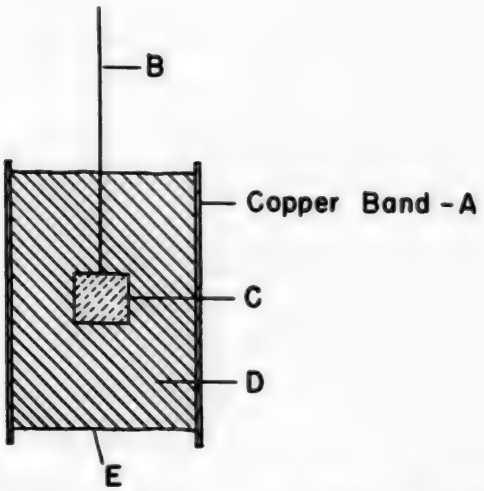

Fig. 5.—Cell for testing electrical conduction. *A*, Copper band; *B*, electrical connection; *C*, amalgam plug; *D*, insulating material; *E*, outer surface.

The results can be seen in Table II. All the cells, both wet and dry, initially had a resistance over 5 megohms. In all but one cell, at the end of 60 hours, there was a marked increase in resistance, the magnitude being over 1,000 megohms. The resistance remained high for most of the cells, although in a few the resistance did decrease. However, in only one of the cells did the resistance reach a value which would actually indicate poor insulating ability (Cell VIII). When this cell was disassembled and carefully studied, there was gross evidence of leakage between the paste and the Lucite molds. Thus the zinc oxide–eugenol paste was being short-circuited by the alternate path of the saline solution. The insulating ability of the material itself was not altered due to absorption of moisture, but rather by this short-circuiting effect.

Zinc oxide–eugenol paste was also studied in a cell similar to the one shown in Fig. 5. Moisture did not render the material conductive. The data previously discussed were reproduced. Thus, it appears that zinc oxide–eugenol does effectively insulate against electric energy under both wet and dry conditions.

The data for the various materials tested are presented in graphic form in Fig. 6. It is apparent that all are effective insulators under the dry conditions, except calcium hydroxide. Zinc phosphate does gain its resistance somewhat more slowly than resin or zinc oxide–eugenol paste.

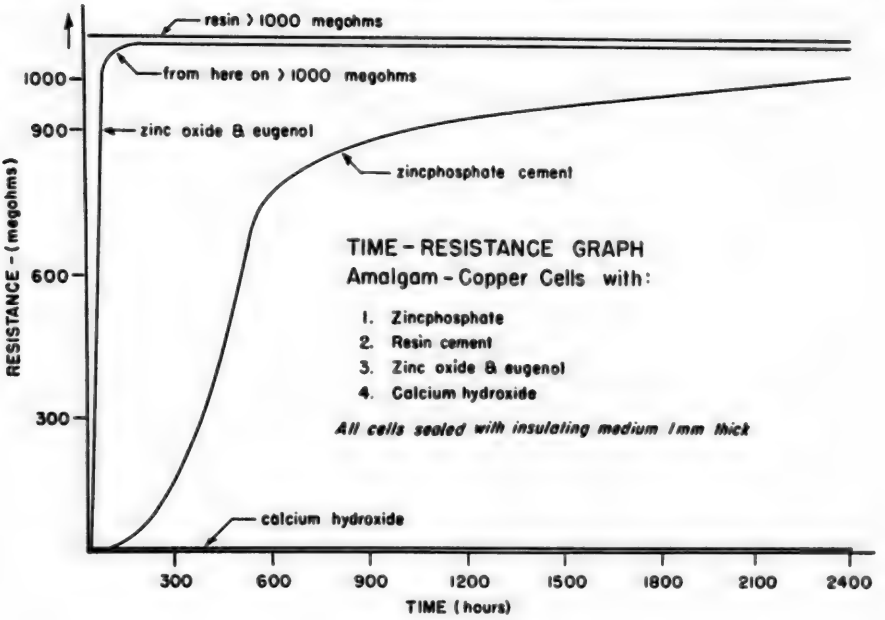

Fig. 6.

DISCUSSION

It is difficult to predict the clinical significance of these data. This investigation has shown clearly that all the commonly used cementing materials, with the exception of calcium hydroxide paste, are excellent electric insulators when maintained in a dry condition. When exposed to moisture, however, zinc phosphate cement becomes a poor insulator, while zinc oxide–eugenol paste and resin maintain a high resistance. Two questions immediately arise. Do the in vitro conditions of these tests, where the margins were exposed to saline solution, correspond to the in vivo cemented restoration, and thus, will the zinc phosphate cement lose its insulating ability due to fluid penetration? Also, is it possible that even though resin and zinc oxide–eugenol are effective insulators when exposed to moisture, the electric current may actually by-pass the cement and go through the dentin? Answers to these questions can be secured only by measurements in the mouth. The test methods that have been established in this research are workable for intraoral measurements, and at the present time, a series of tests are being made on experimental restorations in dogs to correlate the in vivo values with the laboratory data. Only after these tests have been completed will it be possible to predict the actual insulating merits of the various materials.

SUMMARY

Increasing the contact area or the thickness of the cement does not appreciably alter the electric conductivity of zinc phosphate cement when the margins are exposed to saline solution.

The insulating abilities of various materials against electric energy have been investigated under both dry and wet conditions. When maintained dry, all, except calcium hydroxide, are adequate insulators. Acrylic resin and zinc oxide–eugenol paste continued to be effective insulators when exposed to saline solution, while calcium hydroxide and zinc phosphate cement are poor insulators under the wet conditions. Several types of experimental cells substantiated these observations.

The clinical significance of these data cannot be predicted until the in vivo tests now in progress are completed.

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EXPERIMENTAL CAVITY PREPARATIONS IN THE INCISOR OF THE RAT

Effects of Fillings With Paraformaldehyde

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INTRODUCTION

THE purposes of this investigation were: (1) to study the early changes in the dentin and pulp of the incisor of the rat subjected to local mechanical and chemical injury induced by cavity preparation and insertion of different concentrations of paraformaldehyde in a zinc oxide base, and (2) to evaluate the use of the continuously growing incisor of the rat as a test object for the study of the response of the dentin and pulp to various stimuli and irritations.

In view of the greater variability of the pulps of human teeth and their limited availability, the method of employing the rat incisor would facilitate the screening of a large series of fillings and drugs. Since the pulp of the rat incisor is relatively young, and the experimental duration has to be limited to 6 to 8 days owing to the continuous eruption, the final testing of the significant series should be made in human teeth.

The effect of paraformaldehyde on the pulp of the teeth of dogs was previously investigated by Orban[1] and Hord.[2] While experimental studies on the effects of cavity preparation and filling materials have been repeatedly conducted in the teeth of dogs and man,[3-7] no such experiments have been reported in the rat incisor.

METHODS

All animals were anesthetized by an intraperitoneal injection of Nembutal, the dosage being 0.5 c.c. of a 1 per cent solution for 150 grams of body weight. A fresh solution of Nembutal was prepared every 2 days. The composition of 10 c.c. of a 1 per cent solution was: 1 c.c. absolute alcohol, 9 c.c. distilled water, and 0.1 gram Nembutal (Abbott).

Method of Cavity Preparation.—The lower half of the lip of the rat was retracted. With the use of a Bard-Parker scalpel and blade No. 11, an intraoral incision was made in the gingiva, exposing the area of the bone in the vicinity of the mental foramen. Hemorrhage was controlled by a topical application of Adrenalin.

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A cavity was then prepared in the bone with a No. 703 dental bur at the level of the mesial root of the first molar and inferior to the mental foramen, care being taken not to injure the mental nerve (Fig. 1). The cavity was approximately 2 mm. in diameter and 0.75 mm. in depth. The depth was sufficient to expose the lateral surface of the incisor.

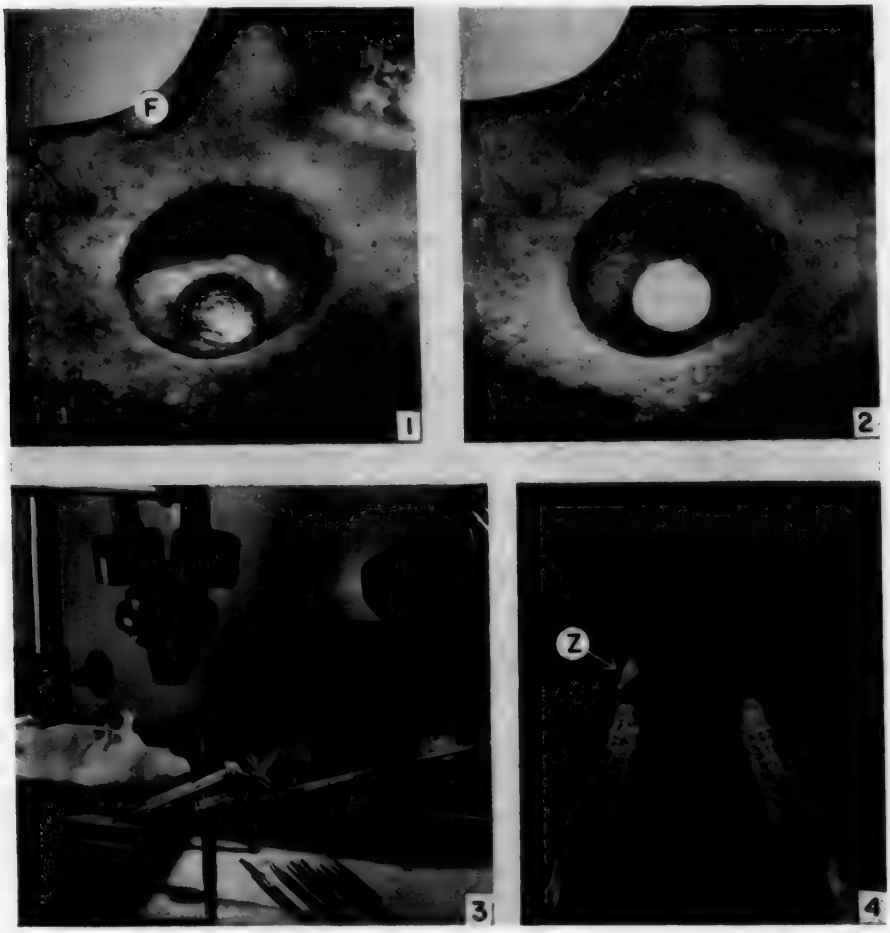


Fig. 1.—Dissected mandible of rat showing experimental cavities in alveolar bone and in underlying dentin of lower incisor. Note relationship of mental foramen (F) to cavities.

Fig. 2.—Dissected mandible of rat showing cavity in alveolar bone after insertion of filling in dentinal cavity.

Fig. 3.—Experimental equipment showing dissecting microscope, light, anesthetized rat on operating table, handpiece of dental engine, and operative instruments.

Fig. 4.—Occlusal roentgenogram of mandible of rat showing filling (Z) in left incisor and alveolar bone. Note 3 molars and extensions of incisors below and beyond these teeth.

Hemorrhage from the injured portion of the periodontal membrane was controlled, and a second cavity, approximately 0.75 mm. in diameter and 0.2 mm. deep, was prepared within the dentin of the incisor. This cavity was

prepared with the use of a No. 701 fissure bur and a No. 33½ inverted cone bur revolving at a speed of approximately 800 revolutions per minute.

The cavity was cleaned with water, dried with air, and filled with the material, which was inserted in the consistency of a paste. The filling was first placed in the incisor and permitted to harden (Fig. 2), and then a separate filling of the same material was placed in the bone. The entire operation was performed with the aid of a binocular dissecting microscope under a magnification of ×9 (Fig. 3) and lasted approximately 30 minutes.

Histologic Methods.—The animals were sacrificed with ether and then decapitated. The mandibles were dissected and radiographed (Fig. 4). Those mandibles which were employed for ground and frozen sections were fixed in 5 per cent neutral formalin. Those planned for celloidin and paraffin sections were fixed in Zenker's formol solution. The stains used were hematoxylin and eosin, silver impregnation, and Mallory's triple connective-tissue stain.

Intravital Staining.—Eight animals were given 1 intraperitoneal injection of 2 c.c. of 1 per cent alizarin red S prior to the operation in order to study the rate of dentin apposition. The teeth of these animals were dissected immediately after sacrifice, fixed in 5 per cent neutral formalin, and ground for histologic study. Ground sections were prepared by embedding the teeth in plaster, cutting slices through the area of the cavity under oil, using the Hauptfuehrer-Spongberg apparatus,[8] and then grinding by hand on an Arkansas stone. Two to 3 sections were ground for each cavity.

MATERIAL

This study is based on histologic sections of the lower incisors of 66 male albino rats 50 to 65 days of age. Cavities were prepared in each animal in the dentin of the mandibular left incisor at the level of the first molar. The untreated mandibular right incisors of 25 of the experimental animals were used as controls.

Zinc Oxide Group.—This group consisted of 33 animals in which the cavities were filled with a zinc oxide base termed "aquadont."[1] The zinc oxide base consisted of the following: zinc oxide—2 parts, zinc sulfate, pulverized—1 part, cornstarch—1 part. This base was selected as a relatively inert material which could be used as a vehicle for paraformaldehyde. Clinical and experimental studies have shown that fillings of zinc oxide and eugenol resulted in minimal pulpal irritation.[3, 4, 7] However, in order to eliminate the possible irritating effect of eugenol, the water-soluble aquadont was selected for this experiment as a further precaution to reduce pulpal injury as much as possible. Aquadont had the added physical advantage of forming a watery paste which hardens rapidly.

Paraformaldehyde Group.—This group consisted of 33 animals in which the cavities were filled with 1 per cent paraformaldehyde ("trioxymethylene," Merck) incorporated in the zinc oxide base. The 1 per cent paraformaldehyde consisted of 99 parts of the zinc oxide base mixed with 1 part of paraformaldehyde.

The animals were sacrificed from 1 to 96 hours following the preparation of the cavities and insertion of the fillings (Tables I and II).

TABLE I. DATA ON SIXTY-SIX EXPERIMENTAL RATS ARRANGED IN POSTOPERATIVE SURVIVAL*

POSTOPERATIVE SURVIVAL (IN HOURS)	NUMBER OF ANIMALS		
	AQUADONT	AQUADONT PLUS PARAFORMALDEHYDE	TOTAL
1	3	3	6
3	3	3	6
6	3	3	6
12	6	6	12
24	6	6	12
48	6	6	12
96	6	6	12
Total	33	33	66

*The untreated mandibular right incisors of 25 of the experimental animals were used as normal controls.

TABLE II. PULPAL CHANGES IN THE RAT INCISOR FOLLOWING CAVITY PREPARATIONS FILLED WITH AQUADONT OR AQUADONT PLUS 1 PER CENT PARAFORMALDEHYDE

		AQUADONT				PLUS 1% PARAFORMALDEHYDE			
		SHALLOW		DEEP		SHALLOW		DEEP	
		DAYS		DAYS		DAYS		DAYS	
	HISTOLOGIC ALTERATIONS	1	2-4	1	2-4	1	2-4	1	2-4
	Hyperemia	+	R	+	R	+	±	+	±
Odontoblasts	Pyknotic nuclei	+	R	+	R	++	R	+++	±
Odontoblasts	Disarrangement and edema				+	++	+	++	+
Odontoblasts	New young odontoblasts					+	++	+	++
Odontoblasts	Increase in subod. cells					+	+	++	+
Odontoblasts	Defective dentin formation								++
	Recovery (R)	Complete				Partial		None	

± Minimal
+ Mild
++ Moderate
+++ Severe

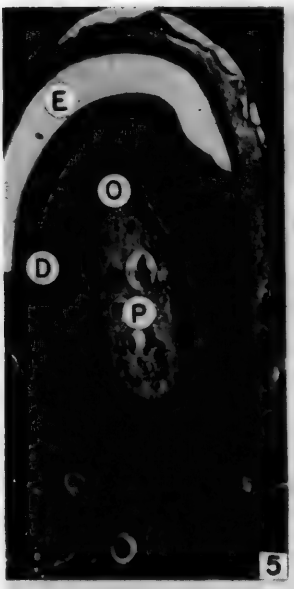
FINDINGS

Normal Controls

This experimental investigation is based upon the changes observed in transverse sections cut at the level immediately distal to the mental foramen, where the odontoblasts are still in their functional stage of activity.

In any given transverse section of the untreated lower right incisors, the peripheral and central portions of the pulp can be readily distinguished (Fig. 5). The peripheral portion consists of the odontoblastic layer and the central portion of the connective-tissue cells and blood vessels. The width of the incisor dentin at the level of the first molar, the area of the cavity, was approximately 325 microns. This amount of dentin, at the normal rate of apposition of 16 microns per 24 hours, took about 20 days to form; thus the odontoblasts in this area were approximately 20 days of age. The dentin showed a regular arrangement of odontoblastic processes which radiate peripherally. The width of the predentin around the entire circumference of the pulp was approximately 10 microns.

At the predentin-odontoblastic border, the terminal bar apparatus of the odontoblasts gave the appearance of a distinct line around the circumference of the pulp. The odontoblasts were regularly arranged. Their cytoplasm



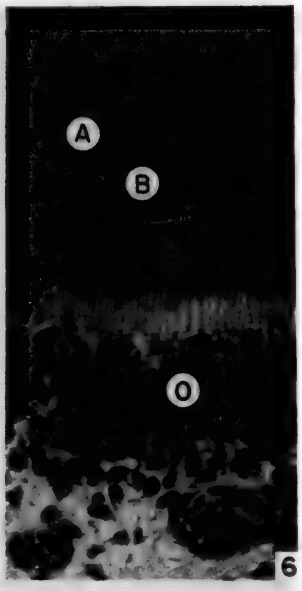


Fig. 5.—Photomicrograph of transverse section of untreated lower right incisor of experimental rat at level of mental foramen. Note normally calcified dentin (*D*); layer of odontoblasts (*O*); enamel space (*E*); and pulp (*P*). Hematoxylin and eosin stain. (×100; reduced ¼.)

Fig. 6.—Photomicrograph showing calciotraumatic response under area of cavity filled with zinc oxide base. Animal was sacrificed after 96 hours. Note hypocalcified layer (*A*); hypercalcified layer (*B*); and normal layer of odontoblasts (*O*). Hematoxylin and eosin stain. (×500; reduced ¼.)

Fig. 7.—Photomicrograph of transverse section through lower left incisor showing cavity which had been filled with zinc oxide base. Animal was sacrificed after 96 hours. Note experimental calciotraumatic response (*C*). Hematoxylin and eosin stain. (×100; reduced ¼.)

Fig. 8.—Photomicrograph of transverse section through lower left incisor showing cavity which had been filled with 1 per cent paraformaldehyde. Animal was sacrificed after 96 hours. Note disturbed and reduced dentin apposition under experimental area. Hematoxylin and eosin stain. (×100; reduced ¼.)

was distinct, and eosinophilic staining showed very fine granules. The width of the odontoblastic layer along the mesial and lateral portions of the tooth was 42 microns. However, the width was about 90 microns along the enamel-covered dentin and about 55 microns along the opposite portion. Between the neighboring odontoblasts and between the odontoblasts and predentin, there was a network of capillaries which was more prominent under the enamel-covered portion of the tooth.

In the central portion of the pulp, 2 types of blood vessels were seen, the large, thin-walled vessels, and the smaller ones which were supported by a connective-tissue lining. Small capillaries were also present. Some of the vessels were filled with red blood cells and others were empty. Occasional lymphocytes and histiocytes were seen.

Experimental Findings

The depth of the cavity was found to be a factor in the degree of the severity of the experimental response. The depth was measured along the length of the remaining dentinal tubules between the floor of the cavity and the underlying pulp. The duration of the survival period was also a factor in the experimental response. The experimental alterations following both the zinc oxide base and the paraformaldehyde fillings were therefore analyzed according to the duration of the survival periods and the depth of the cavity. Those cavities which were more than 100 microns distant from the pulp were considered shallow, and those cavities which were less than 100 microns distant from the pulp were considered deep. In a number of cases, the cavities were not of uniform depth but consisted of shallow and deep portions. The experimental effects were, in such cases, recorded separately for the shallow and deep areas.

The histologic changes for the different survival groups were usually confined to the area corresponding to the zone of injury. The only two alterations found to be located beyond the immediate confines of the experimental injury were: (1) the dilation of the pulpal vessels in the central pulp, which occurred in most cases, and (2) the spread of the calciotraumatic response along a partial or total extent of the dentinal periphery in the specimens filled with paraformaldehyde.

Pulpal Changes Following Cavity Preparations Filled With Zinc Oxide (Aquadont) Base

The experimental effects were minimal and transitory and recovery was complete in 96 hours. Slight dilation of the capillaries in the odontoblastic layer and of the pulpal vessels was observed as early as 1 hour of postoperative survival. This change increased slightly in 24 hours and had disappeared in 96 hours. Fragmentation and pyknosis of odontoblastic nuclei were seen in a few isolated odontoblasts in the 12-hour period only. The dentin increment corresponding to the time of the cavity preparation showed a hypocalcified and a hypercalcified layer, which were confined to the extent of the experi-

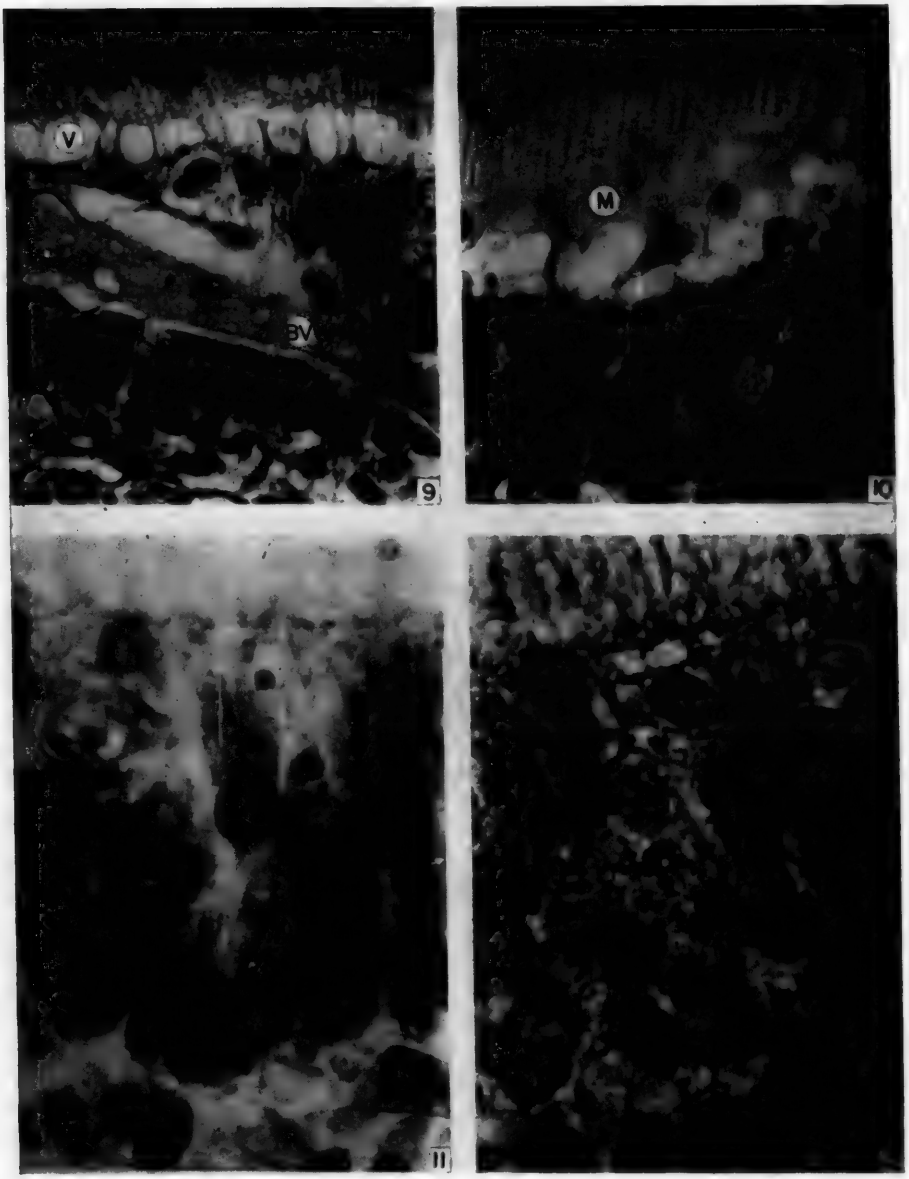


Fig. 9.—Photomicrograph showing vacuoles (*V*) between predentin and odontoblastic layer under area of cavity filled with 1 per cent paraformaldehyde. Animal was sacrificed after 1 hour. Note dilated blood vessel (*BV*). Hematoxylin and eosin stain. (×1,000; reduced ⅓.)

Fig. 10.—Photomicrograph showing serous exudate (*M*) collected in predentin-odontoblastic layer under area of cavity filled with 1 per cent paraformaldehyde. Animal was sacrificed after 12 hours. Hematoxylin and eosin stain. (×1,000; reduced ⅓.)

Fig. 11.—Photomicrograph showing granular appearance of cytoplasm of normal odontoblasts and regular arrangement of these cells. Hematoxylin and eosin stain. (×2,000; reduced ⅓.)

Fig. 12.—Photomicrograph showing increased and coarser granulation in cytoplasm of odontoblasts under area of cavity filled with 1 per cent paraformaldehyde. Animal was sacrificed after 1 hour. Note irregular arrangement of odontoblasts. Hematoxylin and eosin stain. (×2,000; reduced ⅓.)

mental cavity (Fig. 6). This calciotraumatic response constituted a slight accentuation of the normal dentin stratification. It remained as the only permanent record of the experimental interference. Both the decalcified sections (Fig. 7) and the ground sections of the incisors of rats which were given injections of alizarin red S showed a normal rate of dentin apposition.

These findings were essentially the same for the experimental cavities which ranged a distance of 65 to 175 microns from the pulp. Severe alterations occurred in only 1 specimen in which the floor of the cavity was only 15 microns from the pulp. The postexperimental dentin subjacent to the cavity was retarded in apposition and disturbed in calcification, and the odontoblasts were reduced in number.

Pulpal Changes Following Cavity Preparations Filled With Zinc Oxide Base (Aquadont) With 1 Per Cent Paraformaldehyde

First Twelve Hours of Postoperative Survival.—Vacuoles and serous exudate appeared in the predentin-odontoblastic border as early as 1 hour (Fig. 9). The quantity of serous exudate increased up to 12 hours (Fig. 10). Blood vessels showed dilation. The odontoblasts were irregular in arrangement. Their cytoplasm showed granulation which was suggestive of albuminoid degeneration (Figs. 11 and 12). Pyknosis appeared in the odontoblastic nuclei in some of the odontoblasts and increased in incidence in specimens where the cavities were deeper. The terminal bar apparatus of the odontoblasts was broken or absent. The subodontoblastic layer showed an increased influx of cells which appeared to be young fibroblasts. Partial recovery was evidenced in the more regular arrangement of the odontoblasts and the disappearance of the vacuoles in the predentin odontoblastic border.

Twenty-four Hours' Postoperative Survival.—The odontoblastic layer showed an increased number of cells and included a new cell type. This cell was large and had a granular cytoplasm and distinct cell membrane. Its nucleus was pale-staining and vesicular and showed a distinct membrane (Figs. 13 and 14).

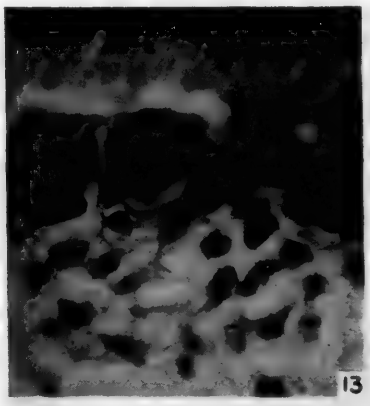
Unlike the teeth filled with zinc oxide base alone, the calciotraumatic response extended along the entire dentinal circumference, although it was more intense subjacent to the cavity.

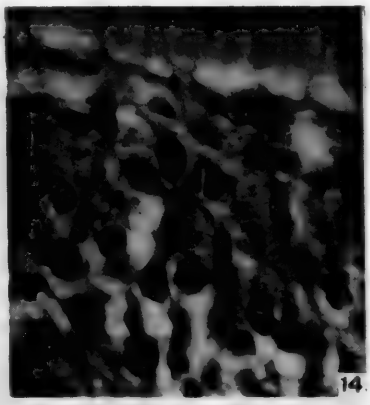
Forty-eight Hours' Postoperative Survival.—The large cells with pale-staining nuclei, first seen in the 24-hour period, increased in number and now constituted the majority of the odontoblastic cells.

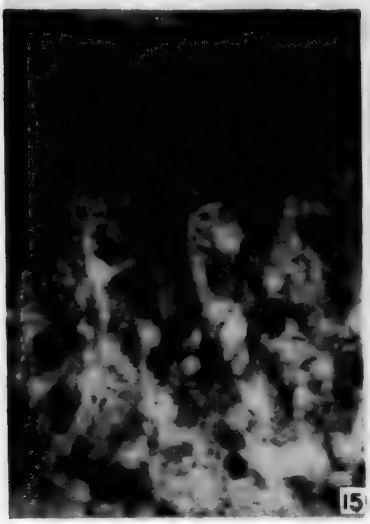
Ninety-six Hours' Postoperative Survival.—The partial recovery first observed in the 24-hour period continued in the specimens with shallow cavity. Recovery was incomplete or lacking in specimens with deeper cavities (Fig. 8). Apposition and calcification of dentin were disturbed. Incomplete attempts of dentin formation were evidenced by dentin projection between the odontoblasts (Figs. 15 and 16).

Summary of Experimental Changes

These changes increased in intensity in the following order of experimental procedure: (1) zinc oxide base in shallow cavities; (2) zinc oxide base in deep cavities; (3) 1 per cent paraformaldehyde in shallow cavities; and (4) 1 per cent paraformaldehyde in deep cavities. The effects of the zinc oxide base in the shallow and deep cavities were mild and transitory.







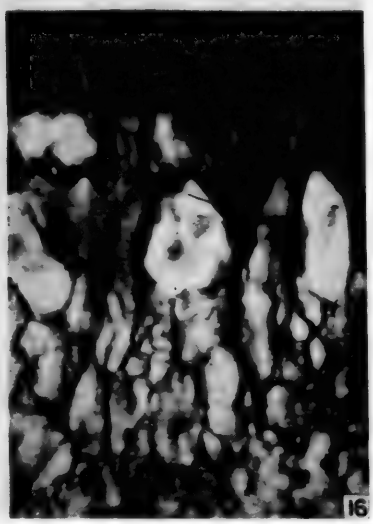


Fig. 13.—Photomicrograph showing layer of odontoblasts in uninjured area of lower incisor. Hematoxylin and eosin stain. (×1,000; reduced ⅓.)

Fig. 14.—Photomicrograph showing layer of odontoblasts under area of cavity filled with 1 per cent paraformaldehyde. Animal was sacrificed after 24 hours. Note increased number of odontoblasts with their large, pale-staining nuclei as compared with those in Fig. 13. Hematoxylin and eosin stain. (×1,000; reduced ⅓.)

Fig. 15.—Photomicrograph showing dentin projections between odontoblasts under area of cavity filled with 1 per cent paraformaldehyde. Animal was sacrificed after 96 hours. Compare with Fig. 18. Mallory's triple connective-tissue stain. (×1,000; reduced ⅓.)

Fig. 16.—Photomicrograph showing dentin projections between odontoblasts under area of cavity filled with 1 per cent paraformaldehyde. Animal was sacrificed after 96 hours. Silver impregnation stain. (×1,000; reduced ⅓.)

Complete recovery was reached in 96 hours. Following the zinc oxide base with 1 per cent paraformaldehyde, only partial recovery was obtained under shallow cavities and none under deep cavities (Table II).

DISCUSSION

The early reaction of the connective tissue and vascular system of the pulp was only slight and may be classified as serous inflammation. The reaction was characterized by hyperemia, a serous exudate, and the mobilization of histiocytes (macrophages). The injury apparently caused an increased permeability of the capillaries which led to serous exudate. However, the injury was not intensive enough to cause migration of leukocytes.

The serous exudate filled the tissue spaces, especially between the surface of the dentin and the odontoblastic perikaryon.[9] This exudate did not coagulate and did not cause the formation of a fibrinous network. The edematous fluid was reabsorbed and removed by blood vessels and lymph vessels, thus leading to recovery. Hamperl and Ribbert[10] pointed out that serous exudate is either absorbed or stimulates the connective-tissue cells to fiber formation. The increase of connective-tissue fibers may lead to a condensation and sclerosis of the tissue. When, in this study, groups of odontoblasts under the influence of paraformaldehyde degenerated and finally necrotized, the pulpal cells proliferated, migrated into the odontoblastic layer, and produced an irregularly arranged network of fibrils.

The absence of an acute inflammatory process observed in this study corroborated similar findings of Orban.[1] He considered the possibility that an acute inflammation might have been present immediately following the injury. In this study, no acute inflammation was observed in animals sacrificed as early as 1 hour after the operation.

Changes in the pulp beyond the area of the experimental cavity, evidenced in this study by a calciotraumatic response around the entire dentinal circumference, were also observed by Orban in his experiments on dogs. Orban found that 1 to 5 per cent paraformaldehyde stimulated the formation of secondary dentin after 3 to 9 months. We found no secondary dentin, probably because of the short survival period of the animals in the present experiment.

Influence of the Depth of the Cavity.—The injury associated with the preparation of the cavity and the filling with a zinc oxide base resulted in the alterations which were transitory and reversible. However, the reaction increased in severity in proportion to the depth of the cavity and its nearness to the pulp.

Sicher[9] suggested that, as in the case of the nerve cells and nerve fibers, the cell body of the odontoblast which is located in the pulp be designated, in contrast to the major odontoblastic process, as the perikaryon of the odontoblast. This distinction leads to an interesting comparison between the cutting of the odontoblastic process and the severing of a nerve fiber. In each case the cytoplasmic process is injured and in each case the effect is increased in

severity the closer the cutting area is to the perikaryon. It is well known that the perikaryon of a neuron shows definite signs of degeneration if its axon is severed sufficiently near the cell body. These degenerative changes, which include swelling of the cytoplasm and chromatolysis (disappearance of Nissl bodies), are, as a rule, reversible if the injury does not occur too close to the perikaryon. If the injury is close to the nucleus, the degenerative changes may become irreversible and lead to the death of the cell.

Similarly, an injury of the dentinal process of the odontoblast may cause degeneration of the entire cell, including the perikaryon, and lead, for example, to the disappearance of the argyrophil granules. These changes may reverse and healing may result if the amputation of the odontoblastic process occurred at a distance from the cell nucleus, as in the most superficial layer of dentin. If, however, the injury occurs closer to the perikaryon of the odontoblast, the damage may become irreversible. In this case, the consequence is necrosis instead of degeneration.

Comparison of Effects of the Zinc Oxide Base Alone and the Zinc Oxide Base With 1 Per Cent Paraformaldehyde.—Our findings confirm the view of Orban[1] that the mildest and least irritating filling available is the base itself. With the zinc oxide base as the common denominator, the difference in the pulpal changes could, therefore, be readily ascribed to the different depths of the cavities.

Since the mechanical procedures relative to the cavity preparations were the same for both types of fillings, provided the cavities were of similar depth, comparison of the effects of zinc oxide base alone and zinc oxide base with paraformaldehyde could be made separately for the shallow and for the deep cavities. A comparison of the effects in both shallow and deep cavities showed that 1 per cent paraformaldehyde resulted in more severe alterations and in delayed or no recovery in contrast to the zinc oxide base alone, which caused only mild and temporary changes. This difference may be ascribed to the paraformaldehyde.

Further evidence of the relative severity of the effects of paraformaldehyde was seen in the generalized calciotraumatic response which occurred only when paraformaldehyde was used. When zinc oxide base alone was used, the effect was sharply localized to the area of the cavity. These facts lead one to assume that the paraformaldehyde diffused beyond the area of the cut tubules into the pulp and affected the neighboring odontoblasts.

It must be pointed out that the effects of the paraformaldehyde per se consisted of an aggravation in the severity of the reaction to injury and did not constitute a different type of reaction.

The response of the odontoblasts thus tends to be stereotyped, and illustrates the general law of specificity of cellular reaction.

Significance of Calciotraumatic Response.—This response constitutes the most immediate reaction to the experimental injury and was observed in every

instance of postoperative survival of 24 hours or longer. It is situated at the dentin level which obtained at the time of the beginning of the experiment, and is readily evident only after additional dentin had become apposed and sufficient time had elapsed for the development of the hypercalcified phase of the calciotraumatic response.

Irving and Weinmann[11] correctly interpreted this response as a reflection of the direct or indirect injury of the odontoblasts. The calciotraumatic response thus reflects the earliest and mildest disturbance in the function of the odontoblast prior to any recognizable morphologic alteration.

The earliest and immediate effect of the experimental injury apparently consists of an alteration in the secretory activity of the odontoblasts which changes the capacity of the dentin matrix to undergo normal formation and calcification.

While the calciotraumatic response is the earliest manifestation, it persists as a permanent record of the initial injury. In fact, in the continued postexperimental apposition and calcification, the calciotraumatic response may become more accentuated by contrast with the preceding and succeeding incremental layers of dentin.

The Value of the Rat Incisor as a Test Object for the Study of Early Effects of Local Injuries.—This investigation confirms the findings of Fish[12] and others that the characteristic tubular pattern of the dentin with its enclosure of the odontoblastic processes offers a unique opportunity for studying the early effects of various mechanical and chemical injuries of these processes upon the odontoblastic perikaryon and adjacent pulpal tissue. Thus early and delicate experimental changes may be analyzed without their being masked by complications that are unavoidable when mechanical or chemical injuries are exerted directly on an opened and exposed pulp.

The dentin of the continuously growing incisor of the rat is especially adapted for an experimental analysis of the effects of different irritants, since it is apposed at a rather rapid daily rate of 16 microns and thus yields a magnified record of response to various stimuli. Furthermore, the rat is an experimental animal which is relatively easily available and easily managed.

The rapid growth of the incisor, however, tends to limit the maximum duration of the experiment, since the effects could be obliterated by the regressive pulpal changes and the eventual attrition of the operated area. Our findings, however, indicate that when the cavity is prepared just posterior to the mental foramen, as was the case in this investigation, the response could be readily traced in survival periods up to 7 days. For a study of longer effects the rat molars would be more suitable.

The early effect of various chemicals upon the odontoblasts and the connective tissue of the pulp can now be tested by incorporating them in the essentially nonirritating zinc oxide base and placing them in dentinal cavities of the rat incisor. Among the chemicals that merit such testing are cortisone, sodium fluoride, and methylcholanthrene.

SUMMARY AND CONCLUSIONS

The effect of cavity preparation and filling with a zinc oxide base (aquadont) or the base plus 1 per cent paraformaldehyde was studied histologically in the lower left incisors of 66 white rats. The animals were sacrificed from 1 to 96 hours after the operation. The unoperated right incisors of 25 of the experimental animals were used as controls. Eight of the experimental animals were given single injections of alizarin red S.

The odontoblasts showed functional and morphologic alterations. The mildest functional changes were reflected in all cases in the calciotraumatic response of the dentin. The morphologic changes, which were seen in a varying number of odontoblasts, ranged from reversible albuminoid degeneration to necrosis, depending upon the depth of the cavities, the nature of the filling material, and the time of the postoperative survival. The necrotic cells were apparently removed by histiocytes and replaced by young pulpal cells. Spikes of dentin matrix formed between the islands of functional odontoblasts in the advanced stages. The pulp reaction could be classified as a serous inflammation characterized by dilation of blood vessels, serous exudate, and mobilization of histiocytes and fibroblasts. Polymorphonuclear leukocytes did not appear.

The lower incisor of the rat proved to be a useful test object for demonstrating the early effects of local mechanical and chemical injuries upon the odontoblasts and upon the pulpal tissue. The method decribed in this investigation is proposed as a biologic screening test for the effects of different chemicals upon the pulpal connective tissue.

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LEPTOTHRIX RACEMOSA IN OPEN CAVITIES

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THE open cavity presents the advanced stages of the caries lesion. Usually the disease process is active and progressive. This results from the larger receptacle for retention of food material which undergoes degradation within the cavity. If fermentable carbohydrates are present, acids are produced. Such acids decalcify dentin and enamel and thus promote advancement of the lesion.

The bottom and side walls of the cavity are covered with a pad or film (Fig. 1) of bacterial material consisting largely of filamentous types of organisms with one end attached to the decaying dentin (Figs. 2 and 3) the other extending outward toward the surface of the pad. At the surface (Fig. 4) there are the growing ends and fruiting heads of the long rods and filaments composing the pad, and also a great variety of other organisms associated with the decomposing food material within the cavity.

The purpose of this paper is to report the frequency of the presence of *Leptothrix racemosa,* based upon finding the fruiting heads of this organism in cavities in a miscellaneous collection of extracted tooth specimens.

MATERIALS AND METHODS

One hundred permanent teeth (75 molars, 16 premolars, 4 canines, and 5 upper first incisors) with large open cavities were selected from a miscellaneous collection of extracted teeth received from extraction clinics in New Orleans, and preserved in 10 per cent formalin. They were rinsed in water, stained in crystal violet (0.5 per cent in water) for about 1 minute or longer, and then rinsed again in water. Under the dissecting microscope, with appropriate lighting from above, material was picked from the bottom or side walls of the cavity, teased out somewhat in a droplet of 50 per cent glycerine (in water) on a slide, and covered with a one-fourth size cover glass.

The method of handling stained tooth specimens and the facilities previously described[1] for another purpose are also quite satisfactory for this purpose. Especially useful for removing material from the tooth for microscopic preparations in studying the composition of the film pad was a teasing needle made by grinding to spade or spatula shape a No. 10 silver steel sewing needle driven (eye end) into a 4-inch length of $\frac{3}{16}$-inch wooden doweling. This instrument was especially useful to run under and lift or dig bacterial film off without breaking it up too much, as occurs when it is scraped off with other instruments. Similarly made teasing needles, ground to very sharp, keen points, were best for teasing apart, under the dissecting microscope, as required, lumps of the film pack, to search for the fruiting heads of *L. racemosa.*

These studies were promoted by facilities to which the author has had access at the School of Medicine, Tulane University of Louisiana, and by aid for equipment and supplies provided by the University.

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Prepared in this way many of the small particles of filamentous material chanced to be mounted so that the part from the outer surface of the pad where the fruiting heads are found (Fig. 5) lay in one direction and the deeper part, which was attached to the tooth, lay in the opposite direction. Many of the rods and filaments lay more or less lengthwise in the preparation. Usually it was not difficult to know which part of a particle represented the outer surface of the pad, when it was in situ. The fact that the pad was

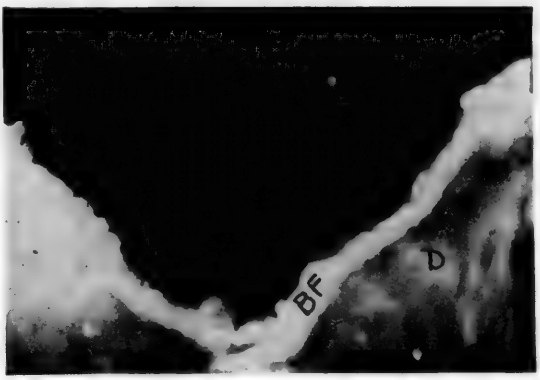

Fig. 1.—Section (unstained, incident lighting) through bottom of dentinal cavity showing heavy bacterial film, *BF*, attached to decaying dentin, *D*.

Fig. 2.—Section (unstained) through side wall of cavity showing heavy bacterial film, *BF*, attached to disintegrating dentin, *D*, and some broken-off loose particles of dentin, *DP*.

composed of filamentous-type organisms was readily observed. If the specimen was stained for only a short period (less than 1 minute) the filaments and fruiting heads at the surface were heavily stained, whereas the filaments and stems were stained less or not at all in the deeper part.

Twenty-five deciduous molars were selected from the same miscellaneous collection and were examined in the same way for fruiting heads of *L. racemosa*.

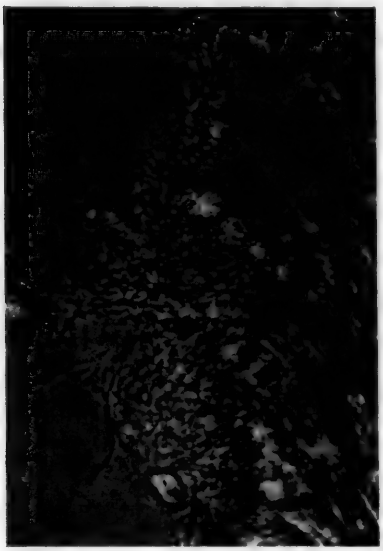

Fig. 3.—Section similar to Fig. 2 except that outer part of bacterial film has been teased off, bringing out filamentous nature of organisms, *FO*, composing the deeper part of the pad.

Fig. 4.—Section of bacterial film in open cavity embedded in egg albumin, thereby retaining growing ends and fruiting heads at the surface, including *L. racemosa*; filaments only deeper in.

Whenever the first preparation from a given specimen in either lot was found negative or unsuitable, a second and sometimes a third was made and examined.

Fig. 5.—Particle of bacterial film from open cavity, prepared by the method described in this paper, showing fruiting heads of *L. racemosa* at the surface side and filaments only deeper in, all the way to the attachment side.

At the time the preparations were made and examined from cavities, preparations were also made and examined from heavy bacterial film on the surface of the tooth. With experience one soon learns to pick material from the surface (depressions and protected locations) where fruiting heads are most abundant and are most likely to be found.

The findings are presented in Table I.

TABLE I. OCCURRENCE OF L. RACEMOSA

TEETH	NUMBER EXAMINED	+ CAVITY + SURFACE	− CAVITY + SURFACE	− CAVITY − SURFACE
Permanent	100	76	14	10
Deciduous	25	21	0	4

RESULTS

Of the 100 permanent teeth, there were 76 in which fruiting heads of *L. racemosa* were found in the cavities and also on the surface. Of the 24 in which no fruiting heads were found in the cavities, heads were found on the surface of 14. Both cavities and surfaces were found negative in 10.

Fruiting heads of *L. racemosa* were found both in the cavities and on the surface of 21 (84 per cent) of the 25 deciduous molars. The 4 with negative cavities also had negative surfaces.

DISCUSSION AND COMMENT

To the author's knowledge, *L. racemosa* has not been isolated in pure culture. Therefore, the rate of growth of individual organisms, the influences that control fruiting and germination of spores and the nature of the metabolic products are unknown. All that can be said at present is that the stems of this organism are present among the different filamentous-type organisms compos-

ing the more or less constant film pack on the surface of teeth, and now also in open cavities. There is no reason to suppose that this organism bears any specific relation to caries, with the exception that it is an important component of the bacterial film (plaque) on teeth and in cavities, without which lesions do not originate or advance.

Viscentini,[2] who first directed attention to this organism, claimed it was present in all mouths. J. Leon Williams[3] stated that "it is common to all mouths which I have examined." Beust[4] recognized wide prevalence of the organism. It was present on most of the hundreds of extracted tooth specimens from miscellaneous sources which the author examined for it.

SUMMARY

Fruiting heads of *L. racemosa* were found in 76 per cent of the open cavities in permanent tooth specimens and in 84 per cent of those in deciduous molars.

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the New York Section

College of Physicians and Surgeons, Columbia University, New York, Oct. 10, 1954

Compiled by Frances Krasnow, *Editor, New York Section*

This was a joint meeting with the Boston and Philadelphia Sections. Although experimental in nature, the attendance was good and interest satisfying. The morning session was chaired by Barnet M. Levy, School of Dental and Oral Surgery, Columbia University, that in the afternoon by Dean R. Nagel, New York University College of Dentistry. Both sessions were summarized by Paul Boyle, University of Pennsylvania School of Dentistry.

The program consisted of: (1) Statistical Methods in the Evaluation of Dental Caries Incidence. *J. W. Fertig, School of Public Health, Columbia University* with discussion by *D. Mainland, New York University.* (2) The Use of Fluorine in the Control of Dental Caries. *D. B. Ast, New York State Department of Health* with discussion by *Frances Krasnow, New York.* (3) The Control of Dental Caries Through Modifications of Tooth Structure. *G. Bevelander, New York University College of Dentistry* with discussion by *R. Sognnaes, Harvard University School of Dental Medicine.* (4) Modification of Oral Flora. *N. B. Williams, University of Pennsylvania School of Dentistry* with discussion by *S. A. Ellison, College of Physicians and Surgeons, Columbia University.* (5) Role of Restorative Dentistry. *H. Addelston, New York University College of Dentistry* with discussion by *Paul Losch, Children's Hospital of Boston.* (6) Modification of Oral Physiology. *Hans Neumann, Columbia University School of Dental and Oral Surgery* with discussion by *Irwin D. Mandel, School of Dental and Oral Surgery, Columbia University.*

New York Academy of Sciences, Dec. 1, 1954

1. Some Considerations on the Classification of the Gram Negative Non-sporulating Anaerobic Bacilli. *Heiner Hoffman, New York University College of Dentistry, New York, N. Y.* The validity of Prevot's classification of the gram negative non-sporulating anaerobic bacilli was questioned, and the present status of the classification in Bergey's *Manual* was discussed in the light of the pertinent investigations in recent years.

2. Electromyography. *Harry Shpuntoff and William Shpuntoff, Jackson Heights, N. Y.* The electromyographic apparatus has been of great value in the understanding of the physiology of centric position. The pitfalls of interpreting an electromyographic record were discussed as well as the use of the myograph in diagnosis and therapy in the various fields of dentistry.

3. A Clinical Evaluation of a Penicillin Dentrifice. *Martin Lunin and Irwin D. Mandel, School of Dental and Oral Surgery, Columbia University, New*

York, N. Y. An experiment was set up in two schools to study the effect on caries incidence of a dentifrice containing 600 units of potassium penicillin per gram of tooth powder. In each school a control and an experimental group were established so as to be similar in sex and age distribution. There was no supervision of tooth-brushing. With no knowledge of whether children were in experimental or control groups, the same individual performed initial and final examinations using bite-wing roentgenograms and mouth mirror and explorer. The dentifrice did not produce a reduction in caries incidence. Acid production, as measured by the Snyder test, was not affected by the dentifrice. There appeared to be no development of a resistance to penicillin by the acidogenic flora or any allergic or toxic manifestations in the group using penicillin.

4. Problems in Evaluating the Snyder Test as an Index of Clinical Caries Susceptibility. *Irwin D. Mandel and Martin Lunin, School of Dental and Oral Surgery, Columbia University, New York, N. Y.* Caries susceptibility, as measured by the Snyder test, was compared with the caries that developed in 76 boys and girls aged 6 to 17 during a one-year period. Using the clinical classifications "negative," "moderate," and "high," we established no significant correlation with the Snyder test estimates. When the Snyder test estimates were correlated with the presence or absence of caries activity, the results were in the borderline zone of statistical significance. Comparison of data from these and other published studies is made difficult by the lack of standardized procedures for estimation of clinical caries activity.

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